Control of flowering time in *Protea* cv. Pink Ice

by
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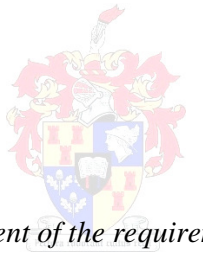

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Declaration

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Summary

Protea cv. Pink Ice is harvested during a low profitability window, from February to May, where less than half the income is realized compared to the high demand and optimal marketing period of December and January. The manipulation of flowering time in 'Pink Ice' would be of great importance not only to increase profits, but also to avoid the high temperature summer months when losses due to sunburn on involucral bracts of the inflorescence are experienced.

In a study which aimed to evaluate the efficacy of an autumn-application of benzyladenine (BA) to *Protea* 'Pink Ice' shoots to advance harvest time, four-flush shoots of mature plants were treated terminally at 500 $mg.L^{-1}$, at both the dormant and greenpoint phenological stages, over ten and eight treatment dates respectively, in the autumn of 2008. Higher percentages of budbreak were achieved with the use of BA compared to that of untreated control shoots, but inflorescence initiation following the completion of a natural or BA-induced autumn-initiated flush, however, did not differ significantly from each other. In dormant shoots, BA promoted the initiation of an additional vegetative flush before winter, although no budbreak could be achieved for the late treatment dates of 22 May and 2 June. The harvest dates of five-flush autumn-initiated inflorescences from January to mid-February were comparable to harvest times observed for six-flush spring-initiated inflorescences. The synchronisation of shoot growth through the use of BA on 'Pink Ice' is therefore recommended to maximise the potential shoots that will budbreak in autumn. Harvests of BA-treated shoots can be advanced compared to that of spring-initiated inflorescences borne on five-flush shoots either by assisting in floral initiation in autumn or by providing an additional flush in spring.

In a second trial the use of pruning and thinning regimes to advance flowering time was explored for 'Pink Ice', where plants were pruned to different numbers of bearers per plant and then thinned to various shoots per bearer. Evaluation of a total number of seven different combinations of bearers:shoots (40:1; 20:2; 13:3; 10:4; 16:2; 12:2 and 24:2) showed that harvests, irrespective of treatment combination, remained spread over a period of 12 months, with average harvest dates contained between 20 March and 14 April in the year following pruning. The percentage of stems harvested before Valentine's Day did not differ significantly between treatments, nor did the percentage of autumn-initiated inflorescences. None of the

bearer to shoot treatment combinations could produce shoots where shoot quality contributed to the significant advancement of flowering time.

Lastly, CPPU (N-phenyl-N'-[2-chloro-4-pyridinyl] urea) as an alternative cytokinin source to BA was investigated for its efficacy to induce inflorescence initiation in *Protea* 'Carnival' and 'Pink Ice' during autumn. Sitofex^{TM}, in a concentration gradient of 1-10 mg.L^{-1}, was applied to both 'Pink Ice' and 'Carnival' shoots on 1 April and 16 May 2008, respectively, whilst BA as MaxCel^{TM} at 500 mg.L^{-1} was applied in April on both cultivars along with a MaxCel^{TM} concentration gradient of 125-750 mg.L^{-1} included in the May treatment date for 'Pink Ice' only. In 'Pink Ice', MaxCel^{TM} applied at 500 mg.L^{-1} together with CPPU at 1 mgL.$^{-1}$ was found to be the most successful treatment in inducing high budbreak percentages of between 70-80% when applied in April. Shoots treated with 1 and 5 mg.L^{-1} CPPU in April induced a significant number of autumn-initiated inflorescences so that 72-81% of shoots were harvested before Valentine's Day. CPPU was, however, ineffective to induce budbreak and thus autumn initiation in both cultivars in May, whilst high budbreak percentages with the April application in 'Carnival' resulted in low or zero percentages of autumn inflorescence initiation in this cultivar. CPPU application both in April or May was unsuccessful to advance flowering time for 'Pink Ice' into the pre-Christmas period.

The manipulation of flowering time in 'Pink Ice' is possible by means of cytokinin application. Further research is warranted into the application of cytokinin in combination with a pruning and thinning regime which can effectively improve plant complexity together with shoot quality in order to achieve harvests for 'Pink Ice' within the pre-Christmas period.

Opsomming

Die natuurlike blomtyd van *Protea* kultivar Pink Ice val saam met 'n nie-winsgewende bemarkingsvenster, vanaf Februarie tot Mei, wanneer slegs die helfte van die inkomste bekom word, in vergelyking met 'n meer optimale bemarkingstyd van Desember en Januarie. Die manipulasie van blomtyd in 'Pink Ice' is van groot belang, nie net om inkomste te verhoog nie, maar ook om die hoë temperature van die somer maande te vermy waartydens groot verliese gely word as gevolg van sonbrand op die omwindselblare van bloeiwyses.

In hierdie studie wat die effektiwiteit van 'n herfsaanwending van bensieladenien (BA) aan *Protea* 'Pink Ice' om blomtyd te vervroeg evalueer, is vier-stuwingslote van volwasse plante terminaal behandel met 500 mgL^{-1}, beide in die dormante en groenpunt fenologiese stadiums, oor tien en agt behandelingsdatums, respektiewelik, in die herfs van 2008. Hoë persentasie knopbreek was verkry met BA-behandelde lote teenoor onbehandelde, kontrole lote, maar bloeiwyse inisiasie wat gevolg het op die voltooiing van 'n natuurlike of BA-geïnduseerde groeistuwing het nie betekenisvol van mekaar verskil nie. In dormante lote was BA in staat om die inisiasie van 'n addisionele groeistuwing voor winter te bevorder, alhoewel geen knopbreek in dormante lote geïnduseer kon word in die laat behandelingsdatums van 22 Mei en 2 Junie nie. Die oesdatums van bloeiwyses soos geïnisieer op vyf-groeistuwingslote in Januarie tot middel Februarie was vergelykbaar met die oestye van lente-geïniseerde bloeiwyses soos gedra op ses-groeistuwingslote. Dus word die sinchronisasie van lootgroei deur die gebruik van BA op 'Pink Ice' aanbeveel om die aantal potensiële lote wat kan knopbreek te optimiseer. Blomtyd kan dus vervroeg word teenoor lente-geïnisieerde bloeiwyses soos gedra op vyf-groeistuwingslote deur blominisiasie in die herfs te induseer of deur 'n addisionele groeistuwing in die lente te besorg.

In 'n tweede studie is die gebruik van snoei- en uitdunningspraktyke om blomtyd te vervoeg vir 'Pink Ice' verken waar plante gesnoei was tot verskillende aantal draers per plant en dan vervolgens uitgedun was tot verskeie aantal lote per draer. Evaluasie van 'n totaal van sewe verskillende kombinasies van draers:lote (40:1; 20:2; 13:3; 10:4; 16:2; 12:2 en 24:2) het getoon dat oeste, ongeag die behandelingskombinasie, steeds versprei was oor 'n periode van 12 maande, met gemiddelde oesdatums beperk tussen 20 Maart en 14 April, in die jaar daaropvolgende waarin snoei toegepas is. Die persentasie lote geoes voor Valentyns

dag het nie betekenisvol van mekaar verskil nie, so ook nie die persentasie lote wat bloeiwyses in die herfs kon inisieer nie. Geen van die draer tot loot behandelingskombinasies kon lote produseer waarvan die loot kwaliteite kon bydra om blomtyd betekenisvol te vervroeg nie.

Laastens, was die effektiwiteit van CPPU (N-feniel-N'-[2-chloro-4-pyridiniel] urea) as 'n alternatiewe sitokinien bron tot BA om blom inisiasie in beide 'Carnival' en 'Pink Ice' gedurende herfs te induseer, bestudeer. Sitofex™ aanwendings was gemaak as 'n konsentrasie reekse van 1-10 $mg.L^{-1}$ aan beide 'Pink Ice' en 'Carnival' lote op 1 April en 16 Mei 2008 onderskeidelik, terwyl BA as MaxCel™ teen 500 $mg.L^{-1}$ aangewend was in April vir beide kultivars, tesame met 'n MaxCel™ konsentrasie reeks van 125-750 $mg.L^{-1}$ in die Mei aanwendingsdatum wat slegs toegedien was op 'Pink Ice'. In 'Pink Ice' is bevind dat MaxCel™ aangewend teen 500 $mg.L^{-1}$ tesame met CPPU teen 1 $mg.L^{-1}$ die mees suksesvolle behandelings was om hoë rusbreek van tussen 70-80% te induseer met die April aanwendingsdatum. Lote behandel met 1 en 5 $mg.L^{-1}$ CPPU in April kon 'n betekenisvolle aantal herfsgeïnduseerde bloeiwyses inisieer sodat 72-81% van die lote voor Valentynsdag geoes kon word. CPPU was oneffektief om knopbreek te induseer en herf-inisiasie te vermag in beide kultivars in Mei, terwyl knopbreking persentasies met die April aanwending in 'Carnival' tot lae of geen inisiasie van herfsgeïnduseerde bloeiwyses gelei het nie. CPPU aanwendings, beide in April en Mei, was onsuksesvol om blomtyd van 'Pink Ice' in die periode direk voor Kersfees te skuif.

Die manipulasie van blomtyd in 'Pink Ice' is moontlik met behulp van die aanwending van sitokiniene. Verdere navorsing is geregverdig, spesifiek met betrekking tot die aanwending van sitokinien in kombinasie met snoei- en uitdunningspraktyke wat effektief plant kompleksiteit tesame met lootkwaliteit kan verbeter om sodoende oestye vir 'Pink Ice' tot in die gesogte periode voor Kersfees te verskuif.

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Our Father up in heaven.

General Introduction

The South African ornamental potted plant- and cut flower industries have the potential to make a substantial contribution to the world floricultural trade (Reinten et al., 2011). Currently South Africa is exporting 76% of all its floricultural produce to Europe, with a smaller market share in the Americas, Asia, the Far East, Middle East and Mediterranean (PPECB, 2012). With the small turnover obtained from domestic markets along with market saturation, South African flower producers continued to shift their focus towards more profitable international markets. Although the percentage of exported flowers is still relatively small, the exploration of the potential of these markets, especially markets that can accommodate niche products, is of great importance (Matthee et al., 2006). With 65% of total floricultural produce exported classified as cut flowers and only 20% as foliage, the South African market share could still be increased when the export of foliage and bouquets is further explored (Bester et al., 2009).

Wild harvests contribute substantially to the South African floricultural industry, with 99 310 hectares of fynbos natural veld being picked (Conradie and Knoetsen, n.d). The commercial production of Proteaceae in South Africa results in a total sum of around 550-850 ha under indigenous species such as *Protea*, *Leucadendron*, *Leucospermum*, *Serruria florida* and *Brunia,* but also including *Chamelaucium* as an Australian native flora (Kotze, 2012). From the cultivated fynbos, in South Africa, 60% are *Protea*, with 17 and 15% being *Leucadendron* and *Leucospermum,* respectively.

Northern hemisphere Proteaceae producing countries have an advantage of being closer to the major floricultural markets and therefore benefit hugely from reduced freight costs compared to more remote countries such as South Africa and Australia. Although off-season supply of cut flowers to the northern hemisphere favours the production of indigenous South African flora, the recent unprecedented increased transport costs together with the perceptions on the high carbon footprint of imported goods, have emerged as new threats in the international trading of South African Fynbos products. This, together with high labour costs and a decline in funding to promote research will impact negatively on the South African flower trade, if not addressed. Still, modern-day interest in the biodiversity of flora and the

growing need for exciting novelty cut flower products is a major advantage in the favour of the South African indigenous floricultural industry and should be exploited.

The maintaining or expansion of the current market share of South African cut flowers, in competition with northern hemisphere fynbos producing countries such as Israel, Portugal and Spain and southern hemisphere countries such as Australia and Chile which produce similar products in the same marketing window, requires a focussed production- and marketing strategy. Several challenges are continuously faced by South African producers. Firstly, fynbos products are required in sufficient quantities for a relatively long marketing period or on specific market demands such as Allerheiligen or over the Christmas period. Secondly, products of high quality, free of physiological disorders such as leaf blackening and bract browning, together with an acceptable vase life, is of critical importance. Thirdly, market requirements must be adhered to, such as a minimum stem length and phyto-sanitary specifications. Finally, competition in the global floriculture industry can be described as being in a constant state of flux. This is largely because of market trends which are often driven by fashion, where some floriculture products are more popular than others, resulting in fluctuations of both the demand for certain varieties and their prices (Matthee et al., 2006). Therefore, the marketing time of niche floral products is extremely price sensitive.

The optimal marketing period for *Protea* as cut flowers to Europe is during their winter, from September to February, when the highest product prices can be obtained (Gerber, 2000). Prices for *Protea* 'Pink Ice' can drop by 50% after the festive season, from January to February and a further 40% from the January price in March (Nieuwoudt and Jacobs, 2010). Only a small selection of species such as *P. cynaroides*, *P. magnifica* and *P. grandiceps* flower within this optimum marketing period, while the majority of species such as *P. compacta* and *P. eximia* may flower only partially in this period, or entirely outside this required period such as *P. neriifolia* (Gerber, 2000). This limited marketing period from September to February offers a major challenge to the South African fynbos producers. The flowering periods of commercially produced cultivars differ from those of the original parental species. Also, most of these hybrids were selected for favourable production traits such as fast growth or long stems, but not necessarily for a favourable flowering time, within the window of September to February. For example, even though *P. magnifica* flowers inside the optimum marketing period, selections made from *P. magnifica* such

as 'Lady Di' (*P. magnifica* x *P. compacta*), 'Pink Velvet' (*P. magnifica* x *P. compacta*), 'Sheila' (*P. magnifica* x *P. burchellii*) and 'Susara' (P. *magnifica* x *P. susannae*) do not flower within this same period. Similar trends were noticed for cultivars selected from *P. compacta* such as 'Pink Ice' (*P. compact* x *P. susannae*) and 'Carnival' (*P. compacta* x *P. neriifolia*) which only commence flowering towards the end of February. 'Brenda' (*P. compacta* x *P. burchellii*) and 'Pink Duke' (*P. compacta* hybrid) also selected from *P. compacta* only flower from end May onwards (Gerber, 2000).

The flowering time of the *P. compacta* selections 'Carnival' and 'Pink Ice' have been the subject of study for several researchers, with the manipulation of flowering time being the main aim (Gerber et al., 1995; Gerber et al., 2001; Greenfield et al., 1994; Hettasch et al., 1997; Nieuwoudt and Jacobs, 2010). Pruning of 'Carnival' during winter within a biennial regime to synchronize vegetative growth improved plant complexity as well as shoot quality, whilst advancing flowering time from April to February. In 'Pink Ice' Nieuwoudt and Jacobs (2010) exploited the plasticity of the flowering habit of 'Pink Ice' by forcing initiation of inflorescences on the autumn flush through a pruning regime, which resulted in some harvests six to eight weeks earlier, in December and January, compared to the normal February to May flowering window. Still, pruning alone was largely unsuccessful to shift flowering time commercially into the pre-Christmas marketing window.

In further studies on *Protea* cv. 'Carnival' manipulation of flowering time was shown possible through out-of-season floral induction, using the exogenous application of benzyladenine to mature shoots during autumn, in conjunction with a biennial pruning system. Flowering time using this technology could be advanced by three months into the pre-Christmas period, compared to normal flowering for natural spring-induced inflorescences that only flowered from mid-February and onwards (Hoffman, 2006; Hoffman et al., 2009). However, the use of this practice has not yet been explored in other *Protea* hybrids.

For the South African Fynbos industry to reach its full potential continuous exports to existing and new international markets is required as this will also result in more employment opportunities along with increased foreign exchange inflows (Matthee et al., 2006). Such an aim can be achieved for *Protea* with the selection of improved cultivars as well as the manipulation of existing hybrids to achieve flowering in high demand periods. Increased market share can only be achieved if the

technology of flowering time manipulation can be within the reach of each South African producer, as the key to obtain the highest possible price per unit lies in delivering a high quality product in a period of high demand.

The aim of this study was thus to attempt the advancement of harvest time to achieve out-of-season flowering through the manipulation of plant complexity (varying number of bearers per plant and the number of shoots per bearer) as well as to evaluate the efficacy of two cytokinin formulations to induce inflorescences out-of-season in autumn. Out-of-season flowering will allow for higher prices to be realised when being exported during the high demand periods to the northern hemisphere markets, especially for widely planted cultivars such as *Protea* 'Pink Ice'.

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TABLE OF CONTENTS

The referencing and formatting style for the individual papers in this thesis are according to the requirements of the Journal of American Society for Horticultural Sciences. Each paper stands as an individual unit, however repetition between chapters that may occur was thus unavoidable.

Literature review: The Manipulation of Inflorescence Initiation in *Protea* as an Indigenous South African Cut Flower Genus

The Manipulation of Inflorescence Initiation in *Protea* as an Indigenous South African Cut Flower Genus

Inflorescence initiation in *Protea* has been studied by various researchers with the aim to manipulate flowering time (Greenfield et al., 1994; Gerber, 2000; Gerber et al., 2001a; Hoffman et al., 2009; Jacobs, 2010; Nieuwoudt and Jacobs, 2010). The vegetative growth habit of flushing in *Protea* shows similarities to that of some tropical and subtropical fruit crops such as citrus, lychee and mango (Hoffman, 2006). As floral initiation in *Protea* may presumably also exhibit comparative traits, the flowering model of *Protea* was compared to that of these tree crops which are better studied and understood than *Protea* itself. Where relevant, reference was also made to the current understanding of the flowering model for annual species. The role of phyto-hormones in the flowering model with specific focus on cytokinin for both annual and perennial species was highlighted.

1. The flowering model with emphasis on annual species as well as perennial tropical and subtropical woody fruit crops

FLOWERING MODEL IN ANNUAL SPECIES. Floral initiation (FI) can be defined as the irreversible commitment of a meristem to produce a flower (Kinet, 1993). In both annual and perennial species FI is controlled by environmental and endogenous stimuli, with juvenility also being of integral importance in the commitment and competence to flower in perennial tree species (Boss et al., 2004).

The flowering models of the annual species *Arabidopsis thaliana* and *Sinapsis alba* are best understood and have contributed significantly to our current hypothesis of the flowering mechanism and pathways (Bernier et al., 1981; Bernier, 1988; Bernier et al., 1993; Mouradov et al., 2002).

Four major pathways have been recognized to be involved in leading to floral induction and initiation. These include the photoperiodic, autonomous, gibberellin (GA) and vernalization pathways (Fig. 1). Flowering is enabled or regulated by the expression of repressors, or actively promoted by endogenous and environmental signals (Boss et al., 2004). For perennial species floral initiation is mainly driven by environmental stimuli in tropical and subtropical species, while temperate species are stimulated autonomously (Wilkie et al., 2008).

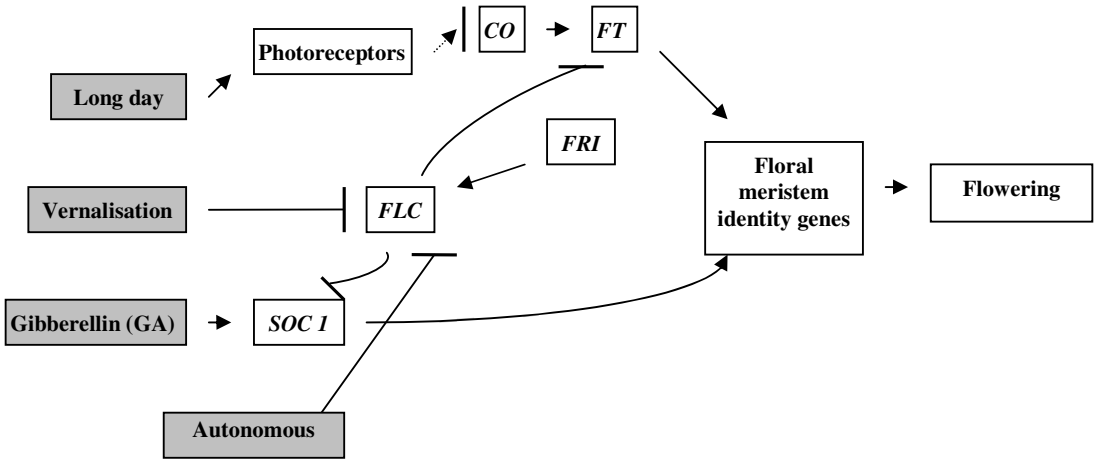

Fig. 1. The floral initiation as for *Arabidopsis thaliana* stimulated by photoreceptors, vernalization, gibberellins and autonomous pathways, working in on the receptive genes, *CO*, *FT*, *FLC*, *FRI* and *SOC1*, either with a positive (presented by pointed arrows) or negative regulation (represented by T- arrows) of flowering (Wilkie et al., 2008).

Bangerth (2009) considered this proposed flowering model to be a basic conserved molecular-genetic model, as a number of genes found to be involved in FI in *A. thaliana* have also been detected in perennial species (Brunner and Nilsson, 2004). The floral stimuli, first believed to be the universal but evasive hormone termed "florigen", are received by the meristem via one of the above mentioned pathways. In *A. thaliana* the protein of flowering *LOCUS T* (*FT*) or the FT-mRNA are now recognised as florigen according to the coincidence model. In the autonomous and vernalization pathways flowering occurs either in response to internal signals such as the production of a fixed number of leaves or to low temperatures. The autonomous pathways act by reducing the expression of the flowering repressor gene *FLOWERING LOCUS* (*FLC*), an inhibitor of *SOC1*, *FT* and *FD* (Searle et al., 2006). *FT*, a small globular protein, forms a complex with *FD*, a basic leucine zipper (bZIP) transcription factor. The *FT/FD* complex activates meristem and floral identity genes such as *SUPPRESSOR OF CONSTANTS 1* (*SOC1*), *APETALA 1* (*AP1*) and *LEAFY* (*LFY*). These genes regulate first the switch to the reproductive meristem and then floral organ identity genes respectively. The

FT protein (Bangerth, 2009; Turnbull, 2011; Wilkie et al., 2008; Zeevaart, 2006) is transported via the phloem (Corbesier et al., 2003) to responsive meristems where it is expressed.

GA levels are often regulated by photoperiod. The accumulation of GA is triggered under short-day inductive conditions when GA_4 is produced in the leaves and transported through the phloem to the apex. GA_4 stimulates the up-regulating of the GA biosynthetic genes, *LFY* and/or *SOC1* in the meristem, leading to floral initiation (Wilkie et al. 2008). Interactions between photoperiodic and GA induction often occur. In *Lolium temulentum* L. endogenous GA accumulates in the meristem following inductive long-day conditions, coinciding with early developmental stages (Turnbull, 2011).

Sugars such as sucrose are transported through the phloem from the source to the sink and may contribute to an inductive signal from the leaves to the apex (Turnbull, 2011). The availability of assimilates in *A. thaliana* and *S. alba* have been observed to increase during long and short-day inductive conditions respectively (Bernier et al., 1998; Lejeune et al., 1993; Turnbull, 2011). The increase in assimilates in the leaves is due to the increase in photosynthesis which is stimulated by mitosis in the apical meristem of induced plants (Bodson and Outlaw, 1985). Sucrose along with cytokinin is considered to be putative signals in flowering via the FT protein and other regulatory pathways (Corbesier et al., 1998; Turnbull, 2011). An increase in the levels of cytokinin within the xylem and phloem is correlated with floral induction conditions which lead to FI (Bernier et al., 1993; Corbesier et al., 2003; Havelange et al., 2000; Lejeune et al., 1993). However, this increase alone is often not sufficient to stimulate the entire process of flowering (Bernier et al., 1993). Cytokinin and carbohydrates which are exported from the roots and mature leaves to stem tops contribute to a complex composition of phloem fluids that may cause floral induction (Davenport, 2003; Lejeune et al., 1988; Lejeune et al., 1993). This increase in cytokinins in the phloem, in the form of zeatin riboside ([9R]2) and isopentenyladenine riboside ([9R]iP), activates a sucrose signal, transported from the shoots to the roots, creating a loop between the shoot, root and shoot (Bernier et al., 1993; Havelange et al., 2000). Cytokinin (iP-forms) is further transported to the apical meristem via the phloem (Bernier et al. 1993; Jacqmard et al. 2002). A concentration increase in cytokinin found in the leaf phloem 16 hours after *A. thaliana* was exposed to an inductive cycle (Bernier et al., 1993; Corbesier et al., 2003)

implicated this phyto-hormone as an integral part of the inductive process of the studied species (Bernier et al., 1993; Jacqmard et al., 2002).

FLOWERING MODEL OF TROPICAL AND SUBTROPICAL SPECIES. The flowering model of *Protea* species shares similarities with that of tropical and subtropical species. In these species both exogenous and endogenous factors play a role in FI (Bangerth, 2009; Wilkie et al., 2008), while flowering of temperate species is mostly endogenously triggered. Similar to *Protea*, citrus, mango and lychee species grow in periodic flushes and flower terminally following a period of dormancy. In subtropical tree species a vegetative flush which is borne on a dormant shoot, will grow actively for approximately two weeks after which it will return to being dormant (Davenport, 2003). For mango and lychee, flowering occurs soon after rapid shoot development (budbreak), following a period of dormancy during which cool temperatures were experienced (Batten and McConchie, 1995; Nûnez-Elisea and Davenport, 1995; Olesen et al., 2002). Frequent flushing without flowering is mainly experienced during high temperature periods (Olesen et al., 2002). Similar results were found for *Protea* cv. Pink Ice (*P. compacta* x *P. susannae*) where vegetative flushing continued during high temperature periods (Bezuidenhout, 2010). Frequent flushing is typical of young trees, but may also be observed in mature trees under conditions of high nitrogen levels, or when supplied with excess water. Shoot development may likewise be promoted by stem pruning, defoliation, foliar nitrogen sprays, and ethylene (Davenport, 2003). The vegetative or reproductive nature of newly formed shoots is hypothesised to be controlled by an interaction of a putative temperature-regulated florigenic promoter (FP) and that of an age-dependent vegetative promoter (VP) most likely a GA, both of which are thought to be located in the leaves (Davenport, 2003). A high ratio of the FP to VP will induce generative shoots, while a high VP to FR ratio induces the formation of vegetative shoots (Davenport, 2003).

The development of a threshold number of vegetative flushes is a determining factor in flower initiation in mango and lychee. It has been reported that inflorescences will only develop on a mature flush or on shoots consisting of one or more flushes (Nûnez-Elisea and Davenport, 1995; Olesen et al., 2002). This correlates with similar findings for *Protea* cv. Carnival (*P. compacta* x *P. neriifolia*) where at least two flushes were considered necessary for flowering. In an annual bearing system, these would generally consist of the autumn flush following harvest and the vigorous spring flush on which floral initiation preferentially takes place.

Occasionally flowering in 'Carnival' may occur on a shoot consisting of a spring flush and its consecutive first summer flush (Greenfield et al., 1994). Limited leaf area and carbohydrates were suggested as cause for the requirement of two flushes and more. For both 'Carnival' and 'Lady Di' (*P. magnifica* x *P. compacta*) the presence of over-wintering leaves was considered essential for inflorescence initiation (Gerber et al., 2002). For 'Sylvia' (*P. eximia* x *P. susannae*) inflorescence initiation could occur on any flush, but flowering followed more readily on the spring flush, if subtended by one or more previous flushes (Gerber et al., 2001a). Following pruning, 'Sylvia' shoots continued to elongate by successive growth flushes until the necessary shoots characteristics such as a critical minimum length or diameter were obtained, after which initiation would occur (Gerber et al., 2001a).

Mango has been observed to initiate inflorescences during periods of cooler temperatures (<18°C) in late autumn and early winter months (Blaikie et al., 2004; Nûnéz-Elisea and Davenport, 1995). The shoot and root growth of lychee alternate with temperatures, where roots grow actively during decreased temperatures under which shoots are dormant (O'Hare and Turnbull, 2004). Cytokinin is known to be synthesized primarily at the root tips (Van Staden and Davey, 1979). Hoffman et al. (2009) associated an increase in cytokinin concentration in *Protea* cv. Carnival immediately prior to budbreak, during periods of low temperature, with floral induction. However, it is unclear what causes initiation in more tropical regions where only a brief period of cooler conditions is experienced. In temperate species such as apple, floral initiation is favoured by an increase in carbohydrate supply due to sufficient light exposure and a subsequent increase in photosynthesis (Wilkie et al., 2008). Mango and lychee, similar to *Protea*, initiate inflorescences terminally only during a part of the growth cycle when new shoots are receptive for floral initiation stimuli (Wilkie et al., 2008). This phase coincides with the elongation of the subtending flushes both for mango and lychee (Batten and McConchie, 1995; Davenport, 2000) as well as in *Protea* (Gerber et al., 2001b; Hoffman et al., 2009). Initiation of inflorescences in autumn months is highly dependent on the maturity of the autumn flush as flowers will not initiate on immature shoots in lychee, but will only initiate after the cool winter inductive period (Wilkie et al., 2008).

2. An overview on the role of phyto-hormones in floral induction and initiation

Floral initiation may be promoted by various phyto-hormones, which may, in a different ratio, formulation or concentration, also lead to the inhibition of flower initiation.

GIBBERELLIN. GA was found to be produced in large quantities by developing seeds of apple, with the highest concentration found during the fruit formation phase, four to six weeks after full bloom (Bubán, 2003). Levels of GA were recorded to be higher in the seeds than in the shoots, leaf and fruit flesh. It was proposed that GA transported from the fruit pedicel inhibits FI, however it is also possible that GA which originates in the meristem tissue may be actively preventing FI (Bubán, 2003).

The GA pathway actively promotes flowering in *Arabidopsis*. For this species GA_4 is produced in the leaves during inductive conditions and transported to the meristems where up regulation of the floral meristem identity gene *LEAFY* (*LFY*) and that of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) as a floral integrator, lead to flowering (Wilkie et al., 2008). GA or *LtFT* thus appear to act as "florigen" in herbaceous species, however flowering is possible without an increase in *LtFT,* as GA can also actively enable the production and transport of other signals required for flowering (Wilkie et al., 2008).

The role of GA in woody perennials is inconsistent for various studies. Substantial evidence has been presented that suggests that endogenous GA inhibits FI in mango, avocado, citrus, sweet cherry and peach, either directly or through the effects of shoot growth (Wilkie et al., 2008). Alternatively, the use of GA biosynthesis inhibitors could improve flowering in mango, lychee and macadamia (Wilkie et al., 2008).

It is well-known that GA stimulates a precocious reproductive switch in gymnosperms. For woody angiosperms exogenously applied gibberellins appear to have a threshold concentration above which flowering is promoted (Meilan, 1997). Crops in which GA promotes flowering at a given concentration include orange, olive and grapevine (Ben-Tal and Erner, 1999; Srinivasan and Mullins, 1978). The biological effect of GA appears to be highly dependent on the type of GA produced or applied, together with the transport rate and the speed at which it is converted to an inactive product (Meilan, 1997).

AUXIN. The role auxin plays in the control of flowering is still not clear. Auxin, believed to have an antagonistic effect to cytokinin, is also affected by factors

such as daylength, temperature and nutrient availability as well as the interaction with other hormonal signals (Müller and Leyser, 2011). As gibberellins stimulated the release of IAA from fruit, IAA may be considered as an alternative signal to gibberellins, where the presence of auxin inhibits flowering (Bubán, 2003). Alternatively auxin may indirectly affect flowering by improving nutritional status and mobilizing carbohydrates. Auxin stimulates vascular tissue differentiation and increases sink strength, therefore increasing the supply of nutrients and hormone, which in turn promotes vegetative development (Meilan, 1997). The presence of auxin is known to stimulate ethylene production which, in turn, is used to promote and synchronize flowering in commercial pineapple production (Wilkie et al., 2008).

CYTOKININ. Cytokinin is known to play an important role in flowering and accumulates in the apical meristem, activating mitosis which is closely linked to the process of flower initiation (Chen, 1985). Endogenous as well as exogenously applied cytokinins are found to promote flower initiation in a number of annual, biennial and perennial species (Bernier et al., 1977; Blanchard and Runkle, 2008; Chang et al., 1999; Chen, 1991; Srinivasan and Mullins, 1978; Yamasaki and Yamashita, 1993; Zieslin et al., 1985).

The exogenous application of cytokinin appears to replace the threshold carbohydrate requirement for flower initiation and inhibits the effect of gibberellins in the annual species *A. thaliana* and some perennial species such as apple (*Malus domestica* Borkh) (Corbesier et al., 2003; Ramirez and Hoad, 1981; Ryugo, 1986). The application of exogenous cytokinin was successful to initiate inflorescences in various species such as *Rosa hybrida* L. (Kapchina-Toteva et al., 2000; Zieslin et al., 1985), *Protea* cv. Carnival (Hoffman et al., 2009), *Vitis vinifera* (Srinivasan and Mullins, 1978), lychee cv. Mauritius (Stern et al., 2003) and *Mangifera indica* L. (Chen, 1985). Cytokinin, however, also has the ability, like gibberellins, to inhibit flowering and therefore has a concentration threshold for exogenous applications where intermediate concentrations may cause vegetative responses, lower concentrations have no effect, but higher concentrations may cause inhibition of inflorescence initiation (Werner et al., 2003).

In *Protea* cv. Carnival Hoffman et al. (2009) found high levels of cytokinin, in the *t*-Zeatin riboside form, present in the xylem sap before and during spring budbreak, to coincide with the time of flower initiation during elongation of the spring flush. This sudden increase in cytokinin concentration in the xylem sap of 'Carnival'

appears to be one of the components in the multifactorial flowering model for *Protea* (Hoffman et al., 2009). In lychee high levels of cytokinin, in the form of zeatin and zeatin riboside, were found to be present during the vegetative growth flush with low levels during the dormant stages, followed by gradually increasing levels of cytokinin throughout flower bud differentiation (Chen, 1991). Furthermore, similar results were found for *Leucospermum* (Napier and Jacobs, 1986) and tuberose corms (Chang et al., 1999) where endogenous cytokinin (zeatin and dihydrozeatin) were recorded to be higher during the floral developmental period than during the vegetative stages.

Endogenous cytokinin production can be stimulated by means of various practices such as root pruning, girdling, bending and water stress. The application of cytokinin exogenously promotes flower initiation, possibly through an effect on the meristem activity (Bangerth, 2006). The timing and application method of exogenous cytokinin is significant in the success of flower initiation, especially when initiation occurs in a certain time of the flush cycle, as for *Protea* (Hoffman et al., 2009).

In other tree crops such as lychee, the exogenous application of cytokinin in the form of zeatin riboside resulted in earlier budbreak when applied to dormant shoots (O'Hare and Turnbull, 2004). However, no significant effect was recorded in terms of flower initiation. In mango, the application of benzyladenine was able to induce flower initiation of up to 80% in buds, resulting in harvests up to two months earlier compared to normal harvest times (Chen, 1985). Hoffman (2006) showed similar results for *Protea* 'Carnival' where three-flush shoots treated with benzyladenine in the autumn months resulted in approximately 90% or more inflorescence initiation, with harvests up to two months earlier than for natural spring initiated inflorescences.

In *Protea,* the timing of the treatment, the position of application as well as shoot characteristics together with flush maturity were important to secure high inflorescence initiation percentages by means of exogenous cytokinin application (Hoffman, 2006). Applications of benzyladenine at 500 $mg.L^{-1}$ to the terminal bud only of mature >7 mm diameter shoots in the dormant or greenpoint stage was recommended to achieve the highest percentage inflorescence initiation.

3. Vegetative and reproductive phenology of *Protea*

VEGETATIVE GROWTH. In *Protea* vegetative flushes sprout from a bearer either in autumn or spring, depending whether an annual or biennial production system are followed. Plants subjected to an annual bearing system will sprout from bearers during early autumn before winter rest and then later again, after a time of dormancy, in spring. After elongation of the spring flush ceases, an inflorescence becomes visible, which will develop subsequently through late spring and summer. Stems cut back at harvest later in autumn towards May, will result in sprouting only after winter bud rest (Greenfield et al., 1994). This single-flush shoot is mostly incapable of flowering. Vegetative development continues by the production of a 1st summer flush, whereafter an inflorescence may or may not initiate.

For a biennial bearing production system pruning in June or July will result in buds sprouting from axillary positions on the bearer only in spring, after a period of bud dormancy. This spring flush is then followed by successive first and second summer- and autumn flushes before growth ceases during the cold period of winter. In the following spring bud burst and vegetative flush elongation follows, a time during which inflorescence initiation will take place. Inflorescences produced in a biennial system are therefore subtended by a shoot consisting of four to five flushes. Inflorescences are rarely initiated on the autumn or second summer flushes (Greenfield et al., 1994), except in *Protea eximia* or hybrids derived from this species.

REPRODUCTIVE GROWTH – TIME OF INITIATION. Time of floral initiation for the various *Protea* species are very different and was categorised into three flower initiating groups by Gerber et al. (2001a). In the first group, inflorescences preferentially initiated on the spring flush for species such as *P. neriifolia, P. compacta* and *P. susannae.* This initiation pattern is also true for hybrid selections from these species, such as 'Carnival' (a *P. neriifolia* hybrid) and 'Pink Ice', which is a cross between *P. compacta* and *P. susannae.* Inflorescence initiation coincides with the elongation of the spring flush (Gerber et al., 2001b) so that by completion of the extension of the spring flush the terminal bud contains an inflorescence primordium which has differentiated into involucral bracts. This differentiation process will produce floral bracts and florets towards the end of spring, before inflorescence growth and development continues into the summer months (Gerber et al., 2001b).

In the second category which includes *P. magnifica* selections such as 'Lady Di' (*P. magnifica* x *P. compacta*) and 'Sheila' (*P. magnifica* x *P. burchellii*), inflorescences are initiated in spring, but only start visual development in early summer (Gerber et al., 2001b). Thus, flower initiation for most cultivars, such as 'Carnival', 'Lady Di' and 'Pink Ice' is limited to spring, following a period of winter rest.

Selections of *P. eximia* and hybrids with *P. eximia* parentage such as 'Sylvia' and 'Cardinal' fall into the third category where inflorescences can be initiated during any time throughout the year. This initiation category therefore has an open window as initiation is not limited to a specific season or flushes (Gerber et al., 2001a). Inflorescences in this category can be initiated during any season, provided four consecutive growth flushes are present or, if initiated in spring, three growth flushes will suffice (Gerber et al., 2001a).

Although the majority of *Protea* initiate inflorescences naturally and preferentially on the spring flush it was shown to be possible to initiate flowers on the autumn flush in 'Pink Ice', by means of a pruning intervention (Nieuwoudt and Jacobs, 2010) or by benzyladenine application in 'Carnival' (Hoffman et al., 2009). These cultivars, derived from *P. compacta, P. neriifolia* and *P. susannae* species that initiate inflorescences on the spring flush, result in harvests ranging from February to May, which is outside the desired period of harvest for optimal marketing. If initiation on the autumn flush can be achieved by means of benzyladenine application in 'Carnival' or pruning in 'Pink Ice' (Hoffman et al., 2009; Nieuwoudt and Jacobs, 2010), anthesis can be reached two months earlier, to fall within the European winter.

REPRODUCTIVE GROWTH – CONTROLLING FACTORS. In *Protea* no single factor has been identified to be responsible as the trigger of floral initiation. However, a threshold level of carbohydrates, a minimum shoot length and thickness, low temperatures and elevated levels of cytokinin have been implicated.

Bezuidenhout (2010) showed that flowering in 'Pink Ice' is advanced with higher temperatures in spring, however when cultivated under supra-optimal temperatures, vegetative production was promoted above reproductive growth. Supra-optimal temperatures (>36℃) therefore have an inhibiting effect on flowering, especially during spring when flower initiation is known to occur. Such high temperatures may cause continuous vegetative flushing as was observed in 'Pink Ice' and therefore either inhibit flowering or delay the initiation of inflorescences to the

first and second summer flush, as opposed to natural initiation on the spring flush (Bezuidenhout, 2010). Furthermore, Bezuidenhout (2010) argued that not only will the higher temperatures promote frequent flushing, but an accelerated vegetative growth rate driven by heat unit accumulation allows for shorter periods between flushes and less opportunity to accumulate sufficient carbohydrate reserve levels to allow for flower initiation. Bezuidenhout (2010) further speculated that the lack of low winter temperatures and, therefore, vernalization, will lead to the possible inhibition of inflorescence initiation. Although temperature is suspected to be a key factor in controlling inflorescence initiation in *Protea*, its influence on flowering would most likely be a function of its interaction with other internal plant factors such as the state of vegetative vigour, juvenility as well as shoot characteristics such as the number of flushes and the thickness of the shoot (Hoffman, 2006). For instance, young vigorously growing shoots which have not yet hardened off were reported to be less effective in achieving inflorescence initiation than more mature shoots (Hoffman, 2006). This was shown to be true when three-flush 'Carnival' shoots responded to treatment with a higher efficacy than shoots consisting of two flushes, for which low inflorescence initiation percentages were recorded (Hoffman, 2006).

The maturity of shoots plays an important role in the perception of floral induction in spring. When mature overwintering leaves were removed six weeks prior to spring budbreak it resulted in no flowering. This phenomenon was ascribed to the removal of sites of inductive signal perception or to the loss of photosynthetic reserves which would have supported floral initiation and inflorescence development (Gerber et al., 2002). However, the development of the inflorescence is not primarily dependent on assimilates as supplied by the overwintering flush, but will preferentially rely on the newly formed spring flush which subtends the inflorescence throughout the development period (Gerber, 2000).

Along with maturity, which can be characterised by an increased dry mass of the flush, the shoot length and thickness were identified to be determining factors in the ability of a stem to initiate an inflorescence. Hoffman (2006) reported that when 'Carnival' shoots were treated with benzyladenine at 500 $mg.L^{-1}$ in autumn for out-of-season flowering, success was limited to thicker shoots (>7 mm) compared to thinner shoots where lower flowering percentages were achieved in response to the treatment (Hoffman, 2006). A threshold stem diameter as well as stem length, implicated a more mature subtending vegetative flush with the capacity to supply photosynthates

required for inflorescence initiation and sustained development (Hoffman, 2006). Similarly to that of 'Carnival', shoot quality in terms of length may be also be a determining factor for flowering in 'Pink Ice' as longer shoots initiated flowers more readily on the autumn flush and reach anthesis early during December and January (Nieuwoudt, 2006).

Furthermore, the presence of an active developing inflorescence as a sink highly decreased the probability of inflorescence initiation on neighbouring vegetative shoots, despite meeting shoot criteria such as length or diameters which are considered requirements for flower initiation (Hoffman, 2006). This initiation inhibiting effect may be due to either hormonal or nutritional factors. Developing structures may produce auxin and gibberellins that may have an inhibiting effect on shoots, leading to failing initiation. Also, developing inflorescences or fruit create sinks which withdraw important metabolites needed for inflorescence initiation (Monselise and Goldschmidt, 1982).

4. Cultural practices that maximise income in *Protea* production

Flowering time and plant complexity in *Protea* were studied by a number of researchers (Gerber et al., 1995; Gerber et al., 2001a; Gerber et al., 2001b; Greenfield et al., 1994; Hettasch et al., 1997; Nieuwoudt and Jacobs, 2010). From these studies a recommendation for a biennial pruning system for the cultivars 'Sylvia', 'Carnival' and 'Pink Ice' emerged. The main aim of these studies was to synchronise shoot growth as well as to advance inflorescence initiation and thus harvest into a more favourable market period. Only synchrony in the growth of shoots could be achieved, with success in advancing harvest of 'Sylvia' into the pre-Christmas period (Gerber et al., 2001a). However only a limited number of 'Pink Ice' stems could be forced to flower within the desired period, irrespective of pruning time (Nieuwoudt and Jacobs, 2010).

As the harvest in these cultivars was not sufficiently advanced by means of pruning, an alternative method was explored whereby benzyladenine application within the biennial pruning system was explored for 'Carnival' to achieve advancement of flowering time (Hoffman et al., 2009; Jacobs, 2010). Shifting of flowering time of up to two months was achieved for this cultivar so that approximately 45% of the harvest was collected before Valentine's Day. An opportunity was thus created to obtain higher prices during this period as prices per

stem for 'Pink Ice' may fall from January to February by approximately 50% and then a further 40% price drop towards the end of March and beyond (Nieuwoudt and Jacobs, 2010).

As most of the commercially harvested species or selections do not flower naturally within this desired marketing period, pruning practices were suggested for a number of key cultivars to improve plant yield and quality and possibly advance the flowering time (Domingues et al., 2010; Greenfield et al., 1994; Gerber et al., 1995; Gerber et al., 2001a; Nieuwoudt and Jacobs, 2010). In addition, the use of benzyladenine to advance flowering, which proved to be more effective than pruning alone in 'Carnival', may also offer a potential strategy in other related cultivars.

4.1 Pruning as a means to manipulate flowering in *Protea*

A pruning system according to biennial bearing was suggested for commercial production in *Protea* cvs. 'Carnival', 'Pink Ice', 'Sylvia' and 'Susara' (Domingues et al., 2010; Greenfield et al. 1994; Gerber et al. 1995; Gerber et al., 2001a; Jacobs, 2010). The biennial pruning system was recommended to improve plant complexity, stem length and subsequently promoting the production of more harvestable stems per plant. Additionally, biennial pruning systems also contribute to a more synchronised harvest which permits a focused marketing strategy by cutting back shoots which already initiated an inflorescence, subsequently creating a more coordinated shoot/stem growth.

ANNUAL vs BIENNIAL PRUNING SYSTEMS. In an annual pruning system *Protea* cultivars such as 'Carnival' produce flowering stems within the January to May harvest period which are cut back to leave a bearer from which new growth will sprout. With harvests in summer or early autumn, an autumn-flush will sprout from the bearer to produce one-flush overwintering shoots. Following budbreak and flush extension in spring, two types of shoots are thus possible on a plant namely: a shoot where initiation of an inflorescence occurs on the spring flush which sprouted from a terminal position or a shoot which fails to initiate an inflorescence on the overwintering shoot, where the spring flush sprouts from an axillary bud position (Jacobs, 2010).

Pruning to cut non-flowering stems and shoots back to bearers of 15 cm in winter would synchronise shoot growth to an almost exclusively vegetative state as all shoots are forced to sprout from an axillary position in spring (Greenfield et al.,

1994). After budbreak in spring and flush succession throughout the growth season, shoots will consist of at least three flushes, namely a spring-, first summer- and second summer-flush and a possible fourth flush in autumn, creating a stronger shoot to facilitate faster inflorescence development and thus the possibility of an advanced harvest time (Jacobs, 2010).

A winter pruning system is managed in an "on-year" and "off-year" system where a production block is divided in two, with each section generating harvests every alternate year. The establishment of such a pruning system should be implemented while plants are still young to allow for greater crop potential (Hettasch et al., 1997).

By subjecting plants to a biennial pruning system synchronised shoot growth is stimulated, producing more marketable stems compared to the annual system and thus compensating for the lack of an annual income (Gerber et al., 1995; Hettasch et al., 1997; Nieuwoudt and Jacobs, 2010). The biennial pruning system as applied to 'Carnival' and 'Pink Ice' resulted in stems longer than 70 cm, whilst a greater plasticity in flowering time of 'Sylvia' and 'Pink Ice' was observed (Gerber et al., 2001a; Greenfield et al., 1994; Nieuwoudt, 2006). The advancement of flowering time of stems in a biennial system can be ascribed to an increase in photosynthetic resources with more subtending flushes and thus increased shoot length and thickness as well as an increased number of leaves on the overwintering shoot which promotes the rate of flowering development (Jacobs, 2010).

The biennial pruning system in 'Carnival' showed an increase in numbers of harvestable stems with an average of 69% more stems harvested when pruned in July to September, compared to the annual pruning system, in March to May with the harvesting cutback (Gerber et al., 1995). When pruning was done between June and September approximately 90% of stems longer than 50 cm were recorded compared to less than 50% of stems of the same length in an annual pruning system (Gerber et al., 1995; Hettasch et al., 1997). Pruning in winter was therefore proposed for *Protea* cv. Carnival to achieve longer, more marketable stems with the additional advancement of harvest maturity peaking in February (Hettasch et al., 1997).

Similar studies were repeated for *Protea* 'Sylvia' where comparative results found that a higher number of harvestable stems as well as longer (>40 cm) stem lengths were attained when shoots were pruned to bearers from June to September (Gerber et al., 2001a). The highest number of harvestable stems was produced when

pruning was scheduled in June. Thus pruning in winter, as for 'Carnival', also resulted in the highest percentage of inflorescences harvested during the high demand period, from September to February (Gerber et al., 2001a). 'Sylvia', unlike 'Pink Ice' and 'Carnival', has the ability to initiate inflorescences at any time during the year and is thus not limited to the spring flush with harvests only from February to May as is the case for 'Carnival' and 'Pink Ice' (Gerber et al., 2001a). Despite this open-window for inflorescence initiation in 'Sylvia', for initiation to proceed a three-flush shoot is required, although initiation can occur on a one or two-flush provided it is subtended by an overwintering shoot (Gerber et al., 2001a).

PRUNING OF PROTEA 'PINK ICE' - FLOWER INITIATION AND FLOWERING TIME.

The possibility of 'Pink Ice' to achieve harvests during the optimal marketing period from September to December or up to the end of January was explored as the initiation of inflorescences during autumn could shift harvests to December and January (Nieuwoudt and Jacobs, 2010). Nieuwoudt (2006) showed that pruning of 'Pink Ice' to bearers in winter resulted in a four- to five-flush shoot in the following autumn. Initiation of inflorescences on four- or five-flush shoot in autumn would provide the benefit of a three months earlier initiation time compared to the natural spring initiation. Autumn-initiated inflorescences that develop during the cooler winter months would require an additional four to six weeks longer development period than spring-initiated inflorescences. Despite this longer development period the harvest would still be up to six weeks earlier than that of naturally spring initiated inflorescences (Nieuwoudt and Jacobs, 2010).

'Pink Ice', in which flower initiation is possible on the spring as well as the autumn flush when managed in a biennial pruning system, will still rarely flower between June and November, irrespective of the pruning date (Nieuwoudt, 2006). Resources in terms of shoot length and number of flushes may be determining factors at the time of inflorescence initiation as longer shoots of an increased diameter will initiate more readily on an autumn flush in 'Pink Ice' (Nieuwoudt, 2006). Pruning and pinching of 'Pink Ice' can be used to improve plant complexity and shoot quality, thereby increasing the number of harvestable stems, average stem lengths as well as influence the time of inflorescence initiation compared to that of un-pruned plants (Nieuwoudt, 2006).

Nieuwoudt (2006) described the failure to initiate inflorescences in 'Pink Ice' in the time period of June to November to be likely due to three possible factors. The

first factor pertains to the sprouting of buds lower down the bearer where they are being overshadowed by more vigorous distal shoots, causing an apical dominance inhibiting effect on growth of shoots lower down on the bearer. Secondly, the development of already initiated inflorescences may compete with non-flowering shoots for assimilates as the young developing inflorescence will be a major sink, resulting in a reduction in growth of non-flowering shoots. Lastly, the shoots lower down on the bearer would grow poorly due to the mentioned overshadowing effect with the distal shoots growing more strongly because of the exposure to sufficient sunlight.

Pruning in late winter to ensure synchronized, vegetative shoot growth for successive and vigorous flush elongation of stems would be the first step in ensuring flower initiation in autumn for 'Pink Ice'.

PRUNING OF PROTEA 'PINK ICE' – IMPORTANCE OF SHOOT QUALITY. Various pruning times for 'Pink Ice' were explored by Nieuwoudt (2006). Pruning to bearers in January and February resulted in most of the shoots flowering in December and January. However, this pruning time produced a significant number of vegetative shoots during the first initiation time, creating an extended flowering period where shoots were harvested over three harvesting periods from March-May, December-May and December-May, respectively (Nieuwoudt, 2006).

March pruning resulted in the highest number of stems harvested during the favourable marketing period within December and January, however pruning in winter (June/July) provided the best commercial option. For this pruning time the highest number of harvestable stems per plant along with the highest number of stems during the optimal marketing period was delivered (Nieuwoudt, 2006). Pruning in winter thus resulted in the highest income per plant with only 4% of stems being shorter than 60 cm and yielding of ±40 flowering stems per plant compared to the 15-20 stems produced in an annual bearing system (Nieuwoudt and Jacobs, 2010).

PRUNING OF PROTEA 'PINK ICE' – BEARERS INFLUENCE ON STEM QUALITY. In addition to time of pruning the length of the bearer may also influence the number of harvestable stems. Longer bearers produced more bud sprouting, while shorter bearers required longer time for budbreak (Nieuwoudt, 2006). However, longer bearers resulted in shorter shoots which resulted in a total reduced leaf area, presumably due to competing for light and a subsequently lower photosynthetic capacity (Nieuwoudt, 2006). Leaving more bearers per plant resulted in poorer shoot

growth with shorter and thinner shoots and subsequently less harvestable stems per plant, or an extended harvesting period, than when bearers were reduced (Nieuwoudt, 2006).

The number of bearers per plant influenced the number of harvestable stems per plant, which in return, regulates the length of stems and therefore the per unit price (Nieuwoudt, 2006). This emphasized the importance of the controlling number of harvestable stems permitted to develop on a bearer (Nieuwoudt, 2006).

4.2 Exogenous application of cytokinin as benzyladenine to manipulate flowering time in *Protea*.

Flower initiation of 'Carnival', similar to that of 'Pink Ice', occurs predominantly on the spring flush, which coincides with the natural increase of cytokinin concentrations in spring (Hoffman et al., 2009). The presence of high concentrations of cytokinin in the xylem in spring was correlated to the initiation of a vigorous vegetative flush and subsequently also to flower initiation during the early phase of the elongation of spring flush. In 'Carnival' success with out-of-season flowering was achieved by means of application of benzyladenine. This was, however, found to be highly dependent on the management of the vegetative growth prior to treatment.

BENZYLADENINE APPLICATION – FLOWER INITIATION AND TIME OF APPLICATION. Inflorescence initiation in *Protea* which occurs during the elongation of the subtending flush (Gerber et al., 2001), coincided with the mentioned increase in cytokinins in the xylem of *Protea* 'Carnival' during the budbreak and elongation of the spring-flush (Hoffman, 2006). The exogenous application of benzyladenine in autumn significantly increased the possibility of 'Carnival' to initiate inflorescences on an autumn-flush, creating out-of-season autumn-initiation (Hoffman et al., 2009). The application of exogenous cytokinins to a shoot prior to winter may increase the inadequate levels of cytokinins responsible for the inability to initiate inflorescence on an autumn-flush in its natural state (Hoffman et al., 2009).

TIME, CONCENTRATION AND STAGE OF APPLICATION. The success of treatment with benzyladenine to achieve inflorescence initiation is highly dependent on the time and position of application on the shoot together with the concentration of the application and the quality of shoot being treated.

Treatments done later in autumn (May) resulted in higher percentages of inflorescence initiation whereas earlier treatments in March yielded limited success (Hoffman et al., 2009). Increasing the concentration of exogenously applied cytokinin did not compensate for the lack of reserves that may have been present early in the season or when treating a shoot of inferior quality (Hoffman, 2006). The stem diameter and leaf number were considered determining factors in the efficacy of benzyladenine treatments to achieve inflorescence initiation outside of the normal initiation period. This was shown to be true when two-flush shoots were not successful in initiating inflorescences at the same significant rates compared to those of three-flush shoots, having an additional more mature subtending flush (Hoffman, 2006). Treatment with of benzyladenine applied at 50-500mgL^{-1} to terminal buds only as compared to the entire shoot length of 'Carnival' in April, led to higher incidences of inflorescence initiation in dormant shoots than shoots sprouting naturally during this time of the year (Hoffman et al., 2009). Exogenously applied cytokinin at 500 mg.L^{-1} was thus found to assist in budbreak, followed by initiation of inflorescences in 'Carnival'. However higher concentrations of BA, exceeding that of 500 mg.L^{-1}, did not significantly increase the inflorescence initiation percentages (Hoffman, 2006).

BA applications to the terminal bud specifically of a mature three-flush shoot in the dormant and greenpoint stages were found to be most successful (Hoffman, 2006). Application later during flush development such as the elongation stages I and II either resulted in lower or no inflorescence initiation (Hoffman, 2006). Thus it was concluded that the growth stage of the elongating shoot as well as the method of application were important to ensure high efficacy of benzyladenine to induce flowering in 'Carnival'.

HARVEST ADVANCEMENT. The inflorescence initiation in autumn induced by BA application was able to advance harvest times by four months compared to that of the naturally spring-initiated inflorescences (Hoffman et al., 2009). Harvest times of cytokinin-assisted inflorescences of 'Carnival' concluded by early December compared to the harvest of spring-initiated inflorescences which spread from end January to end March. The inflorescences produced on shoots treated with BA were found to have more involucral bracts, higher numbers of florets, a more intense colour and a larger inflorescence than the naturally initiated inflorescences (Hoffman, 2006).

No increased occurrence of leaf blackening was recorded with BA-induced autumn inflorescences.

5. Conclusion

Although the flowering model in *Protea* is still not well understood, it appears that *Protea* has plasticity in bearing to some extent, which can be exploited by pruning as a means to manipulate flowering time. The use of growth regulators, especially gibberellins and cytokinin, appear to have both inhibiting and promotive effects on floral initiation in woody perennials. For *Protea* cv. Carnival the application of exogenous cytokinin was highly effective to induce inflorescences out of season, provided application specifications were followed. The application of cytokinin in this hybrid resulted in an advanced harvest by as much as three months and could be successfully used to manipulate flowering time into the pre-Christmas period. Inflorescence initiation success in 'Carnival', however, depended on a combination of factors such as the flush maturity, the number of leaves, shoot diameter, the number of flushes subtending the terminal bud as well as the time of treatment during the season together with the prevailing temperature at the time of application. Similarly, shoot characteristics were also reported to facilitate natural autumn inflorescence initiation of 'Pink Ice'. Manipulation of vegetative growth and shoot synchrony by means of pruning resulted in an advancement of harvest (6 weeks) in 'Pink Ice', although only in low percentages of the total number of harvested stems.

In this study the use of cytokinin and pruning to manipulate plant complexity in 'Pink Ice' will be explored to advance flowering time as the potential of these technologies could significantly impact on production systems to deliver 'Pink Ice' shoots with advanced harvest times, possibly in the pre-Christmas period.

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Paper I: Manipulation of Vegetative Growth in *Protea* cv. Pink Ice by Means of Autumn Application of Benzyladenine to Achieve Advanced Flowering Time

Manipulation of Vegetative Growth in *Protea* cv. Pink Ice by Means of Autumn Application of Benzyladenine to Achieve Advanced Flowering Time

ADDITIONAL INDEX WORDS: Shoot synchronization; budbreak; inflorescence initiation; cytokinin

ABSTRACT. Marketing a floricultural product in a period of high demand is of great importance to maximize profit share. Such a prime marketing window for South African-produced *Protea* is largely limited to the European winter months of September to January, which also includes the Christmas period, a time when higher prices can be obtained. In the southern hemisphere *Protea* cv. 'Pink Ice' naturally flowers from February to May as floral initiation predominantly occurs on the spring flush. However 'Pink Ice' has an inherent plasticity in its time of floral initiation and bearing habit which can be manipulated by means of synchronized pruning in a biennial production system. Still, only a limited proportion of the harvest could be shifted into the highly required December to January marketing window, using this technique.

The use of Benzyladenine (BA) to induce out-of-season inflorescence initiation was shown to be successful when applied to *Protea* cv. 'Carnival' shoots in autumn and allowed for harvest advancements of up to 3-4 months. This study aims to evaluate the efficacy of an autumn-application of Benzyladenine to *Protea* 'Pink Ice' shoots to advance harvest time of this widely-planted *Protea* cultivar. Four-flush 'Pink Ice' shoots were treated terminally at 500 mg.L^{-1}, in both the dormant and greenpoint phenological stages, over ten and eight treatment dates respectively, in autumn of 2008.

The treatment of four-flush shoots promoted the initiation of an additional vegetative flush before winter and therefore significantly decreased the incidences of five-flush spring-initiated inflorescences. Higher percentages of budbreak were achieved with the use of BA compared to that of untreated control shoots. Inflorescence initiation following the completion of a natural or BA-induced autumn-initiated flush, however, did not differ significantly from each other. No budbreak could be achieved for the late treatment dates of 22 May and 2 June. The harvest dates of five-flush autumn-initiated inflorescences from January to mid-February were comparable to harvest times observed for six-flush spring-initiated inflorescences that resulted from a BA treatment in autumn. The synchronisation of shoot growth through the use of BA on 'Pink Ice' is recommended to maximise the potential shoots that will budbreak in autumn. Flowering time could be advanced either by assisting in floral initiation in autumn or to provide an additional flush in spring, thereby advancing floral development significantly compared to spring-initiated inflorescences borne on 5-flush shoots.

Introduction

South Africa is one of the major *Protea* producing countries that aim to export to profitable European markets within their winter months of September to January, a time when local flower production is limited (Coetzee and Littlejohn, 2001). Delivering a high quality *Protea* cut stem within a prime marketing window is essential for southern hemisphere producing countries to maximize their profit share, as high freight cost to long distance markets significantly lowers the profit margin, and subsequently their competitive edge. In addition, for these exporting countries, long-term cold storage poses an increased risk of postharvest disorders such as leaf blackening and chilling injury, in comparison to northern hemisphere competitors such as Israel, the Canary Islands and Portugal for whom long-term storage to the same markets is not required.

Only a selected number of commercially grown *Protea* species and cultivars such as *P. cynaroides, P. magnifica* or 'Sylvia' (*P. eximia* x *P. susannae*) flower naturally in late spring to early summer (August to November) in the Western Cape (Gerber, 2000). However, the widely planted, productive *Protea* hybrid 'Pink Ice' (*P. compacta* x *P. susannae*) as well as the more exclusive cultivar 'Carnival' (*P. compacta* x *P. neriifolia*) only flower late summer to early autumn (February to May), together with other popular cultivars such 'Brenda' (*P. compacta* x *P. burchellii*) and 'Susara' (*P. magnifica* x *P. susannae*). This flowering time coincides with the time when the demand for *Protea* from the European markets is generally low (Gerber, 2000).

Manipulations to advance flowering time in *Protea* through the exploitation of a plasticity in the timing of floral initiation by means of pruning practices were studied by Greenfield et al. (1994), Gerber et al. (1995), Gerber et al. (2001a), Hettasch et al. (1997) and Nieuwoudt (2006), in a range of cultivars such as 'Carnival', 'Sylvia' and 'Pink Ice'. However, only the flowering time of 'Sylvia' could commercially viably be shifted into the much-valued pre-Christmas period (Gerber et al., 2001a). This success can most probably be ascribed to the inherent ability of 'Sylvia' to induce inflorescences on any four-flush shoot, irrespective of the season (Gerber, 2000). For the other mentioned cultivars, the majority of stems were still harvested only towards late January or even after Valentine's Day.

Earlier harvest of these cultivars may not only hold prospects for premium prices in a higher demand period, but may also suffer much less damage to sunburn of

the involucral bracts of the inflorescence as advanced harvests will avoid the extremely hot summer months of January to March, especially in warmer production areas.

Protea cv. 'Pink Ice' almost exclusively initiates inflorescences terminally on the spring flush and reaches anthesis during January to May (Gerber, 2000). However, studies by Nieuwoudt (2006) showed that under a pruning regime floral initiation in 'Pink Ice' is not limited to only the spring flush, as this cultivar can initiate inflorescences either on an autumn or spring flush (Nieuwoudt and Jacobs, 2010). Inflorescence initiation on the autumn flush may advance flowering as early as December, as opposed to a flowering window of February to May, when floral initiation is confined to the spring flush. Critical to the strategy of advancing flowering time through pruning, Nieuwoudt (2006) suggested the use of a biennial pruning system to achieve the required shoot synchronisation.

Implementation of a biennial pruning system in 'Pink Ice', similar to that recommended by Greenfield et al. (1994) for *Protea* 'Carnival', resulted in advanced harvests by 4-6 weeks. Such a biennial pruning system implies a harvest every other year, where the orchard is divided into alternating blocks to yield an "off" and "on-year" with plants producing vegetative growth or being in the reproductive phase, respectively. Plants are effectively pruned in winter whereby the flower crop of the following autumn is forfeited (Nieuwoudt and Jacobs, 2010).

Pruning of *Protea* plants on a monthly basis was performed by both Gerber et al. (1995) and Nieuwoudt and Jacobs (2010) for 'Carnival' and 'Pink Ice', respectively. Similar results were obtained in both studies where pruning in June to July yielded the best income per plant, mainly due to a higher number of harvestable stems than what could be obtained from the combined yield of two consecutive crops from plants of which shoot growth is not synchronized. In addition, this biennial pruning-based management system has been shown to be more effective in producing better quality cut flower products of longer stem length from which potentially higher prices can be obtained, compared to stems produced in an annual pruning system. When 'Pink Ice' plants were pruned by Nieuwoudt (2006) in February or March, the majority of the flowering stems were harvested during December and January. However, the yield of this pruning date was much lower than that for stems that sprouted from bearers pruned in June or July. Therefore, despite synchronisation by means of pruning, 'Pink Ice' continued to induce inflorescences more readily on a

spring flush than an autumn flush, as was reflected in the higher number of stems being harvested between February to May, compared to December to January. Flowering rarely occurred in the June to November period, irrespective of the month of pruning. Nieuwoudt and Jacobs (2010) concluded that pruning as a horticultural manipulation tool is not effective to commercially advance the harvest time of the majority of the 'Pink Ice' stems into the desired pre-Christmas period. The study of Nieuwoudt and Jacobs (2010) further highlighted the determining role of shoot length in May to the propensity of the shoot to flower in the following season.

The system of inflorescence initiation in 'Pink Ice' can be compared to that of 'Carnival', as both cultivars naturally initiate inflorescences on the spring growth flush that follows after a period of winter rest. Inflorescence development continues through to anthesis in approximately the same period of January/February to May. Hoffman et al. (2009) were successful in inducing budbreak and inflorescence initiation on the autumn flush in 'Carnival' by the application of 500 $mg.L^{-1}$ benzyladenine (BA) to dormant, terminal buds on three-flush, mature shoots. Flowering percentages on the autumn-flush of as high as 90% were achieved by means of BA application and flowering times were advanced by approximately two months. As a consequence, a significant proportion of production was shifted into the high demand period of November to January, compared to that of the natural spring-initiated inflorescences which were only harvested from February to May (Hoffman, 2006). Although this technique has been successful for 'Carnival', to date no such system has been attempted for 'Pink Ice'.

The aim of this study was to evaluate the efficacy of an autumn-application of benzyladenine on terminal buds of four-flush *Protea* cv. Pink Ice shoots to stimulate an additional vegetative flush prior to winter that will allow for out-of-season inflorescence initiation in autumn. The potential advantage of an additional BA-assisted vegetative flush in autumn to promote and advance spring-initiated inflorescence development is also addressed. Both autumn-induced inflorescences as well as spring-initiated inflorescences on shoots with additional pre-winter flushes may be able to achieve advanced flowering times into the sought-after Christmas or pre-Valentine's period.

Materials and Methods

EXPERIMENTAL SITE AND PLANT MATERIAL. Shoots of seven-year-old *Protea* cv. Pink Ice plants (*P. compacta* R.Br. x *P. susannae* Phill.) within a commercial orchard in the Hopefield district (33°2′S 18°20′E) at altitude of 31 m, on the west coast of South Africa, were used as experimental material. The average annual rainfall of approximately 250-300 mm is mainly concentrated in the winter months, from June to August. The orchard was established on a sandy soil, with plants spaced in double rows, 3m wide and 1m between plants. Plants were drip irrigated, and were managed according to established commercial cultivation practices. All plants were pruned in June 2007 according to a biennial bearing system where the orchard was divided into "on-year" (flowering) and "off-year" (vegetative) blocks (Gerber et al., 1995; Nieuwoudt, 2006). All shoots used in this trial were selected from plants in the "off-year" cycle. Plant complexity was pruned to approximately 20 basal bearers to yield roughly 40 flowering stems per plant. Excess buds sprouting from lower auxiliary positions were hand-thinned during mid-October 2007 to a system of two shoots per bearer.

BENZYLADENINE (BA) APPLICATION. Benzyladenine solutions were prepared by diluting MaxCel^{TM} (with active ingredient 1.9 g.L^{-1} 6-benzyladenine; Valent Biosciences Corporation, Libertyville, Illinois) to a concentration of 500 mg.L^{-1} (Hoffman, 2006). Applications were made to the terminal buds of mature four-flush 'Pink Ice' shoots, when the terminal bud displayed either one of two phenological stages. These stages represented the dormant stage when no active growth was visible and where the bud scales were observed to be dry and contracted (Fig. 1A) or the "green-point" stage with the first visible appearance of budbreak, when the terminal bud was displayed as a compact torpedo shaped structure, with a final length of not more than 1cm (Fig. 1B). Solutions were applied weekly by means of a paint brush on ten consecutive dates in the autumn of 2008, namely on 27 March; 3, 10, 17, 24 and 30 April; 8, 13 and 22 May as well as on 2 June. Four-flush shoots were used at each treatment date and were replicated five times in a randomised complete block design, with ten replicates per block. An untreated control replication was included at the first treatment date in March 2008.

BASE-LINE DATA COLLECTION. Prior to treatment each individual stem diameter (mm) was measured directly above the upper intercalation, between the flush subtending the terminal bud and the consecutive flush, using a digital calliper. In

addition, a pooled sample of three leaves located five cm below the terminal bud was sampled on the date of treatment from each stem to determine the respective leaf area (cm^2) and dry weight (g). Leaf area was recorded by means of a digital leaf area meter (Portable Area meter, Li-3000A, Li-Cor, Lincoln, Nebraska, USA), whereas dry weight was obtained by drying leaves in a draught-oven at 60 °C for approximately 24 hours.

PHENOLOGICAL ASSESSMENT. The progression of the vegetative phenology was monitored, starting a week following treatment until visible inflorescence differentiation (Fig. 1C). Inflorescence diameters (mm) were thereafter measured on a biweekly basis from visible differentiation until flowering stems were harvested at the commercial "soft tip" harvest stage, using a digital calliper. The harvest dates were collected to construct a harvest distribution diagram.

At harvest, all flowering stems were grouped into one of three classes according to their response to the benzyladenine application in autumn (Fig. 2). Shoots were categorised according to the time of flush initiation after treatment as well as the number of flushes that was required before inflorescence initiation was achieved. All four-flush shoots at the time of application consisted of a terminal late-summer flush subtended by a mid-summer, early-summer and a spring basal flush. The three categories included: firstly, an autumn-initiated inflorescence on a five-flush shoot. Treatment of a four-flush shoot in this category resulted in a cytokinin-induced, vegetative flush and subsequently an inflorescence initiating in autumn directly following flush elongation (Fig. 2A); secondly, a spring-initiated inflorescence borne on a spring-flush produced at spring budbreak, on a six-flush shoot. The spring-flush in this category is therefore subtended by an autumn flush induced directly after the BA treatment (Fig. 2B); and thirdly, a spring-initiated inflorescence borne on a spring flush, which terminates on a five-flush shoot, but is not subtended by a BA-induced autumn flush (Fig. 2C).

INFLORESCENCE AND SUBTENDING FLUSH CHARACTERISTICS. Five flowering stems from each treatment date were harvested in January 2009 at the commercial "soft tip stage" to assess both the inflorescence quality and the vegetative flush characteristics subtending the inflorescence at harvest. Inflorescence quality assessments included the determination of the number of involucral bracts as well as the inflorescence length (mm) measured from the involucral receptacle to involucral bract tips; the basal diameter (mm) and dry weight (g). Colour measurements on

inflorescences were performed using a colour-guide 45% chromometer and chromaticity coordinate, a* (red-green range) was determined with the use of the CIELab Colorimetric space (Minolta, 1992 Cat. no 6805, BYK-Gordner, USA). Colour readings were taken at the upper part of the bract, with ten replications.

Characteristics of the flush subtending the inflorescence were quantified in terms of stem diameter, leaf area and shoot dry weight. The stem diameter, measured directly below the inflorescence and at the intercalation of the terminal and subterminal flush, was determined using a digital calliper. All leaves of the subtending flush were used to determine the collective leaf area (cm^2) of the shoot by using a portable area meter (Li-3000A, Li-Cor, Lincoln, Nebraska, USA), whereafter leaves were dried together with the stem at 60°C for approximately 48 hours using a forced circulation incubator (FSIE 16, Labcon (Pty) Ltd), to determine the dry weight (g) of each shoot.

STATISTICAL ANALYSIS. Analysis of variance was conducted using the PROC GLM procedure (version 9.1; SAS Institute, Cary, NC) and contrasts were fitted where appropriate. Log transformations were done for data presenting budbreak and flowering percentages. CORR PROC procedure (version 9.1; SAS Institute, Cary, NC) was used to determine R^2 and *P*-values of correlations.

Results

BUDBREAK. Higher budbreak percentage for 'Pink Ice' shoots treated at the dormant growth stage with benzyladenine at the end of March was observed before the onset of winter compared to untreated control shoots (Fig. 3A-3B). By 2 June control shoots recorded a cumulative budbreak of 74% as opposed to 94% recorded for treated shoots, selected on the same date (Fig. 3A-3B). Comparing budbreak incidences of untreated control shoots with that of BA-treated shoots on the same date, the shoots selected on 27 March showed a natural budbreak of 34.69% after a week compared to a 58% budbreak of treated shoots that was recorded over the same period (Fig. 3B). Budbreak was completed within three weeks for both control and treated shoots, but a much steeper initial rate of budbreak incidence was observed for treated shoots (Fig. 3A).

High budbreak incidences were recorded for dormant BA-treated shoots throughout the first five treatment dates up to 24 April (Fig. 4). These percentages were significantly reduced to 44% and lower in the late autumn treatments of 30 April

to 2 June, according to a quadratic relationship. No BA-assisted autumn budbreak could be achieved after 22 May (Fig. 4).

HARVEST DISTRIBUTION AND ADVANCEMENT OF FLOWERING TIME. Harvest dates for inflorescences picked from the commercial blocks ranged from 28 November 2008 to 29 June 2009, with a mean harvest date of 23 March 2009 ±37 days (Table 1). Harvest peaks (>15%) shown for all BA-treated shoots, irrespective of treatment dates and phenological stages, peaked prior to the mean commercial harvest of 23 March (Table 1).

Treatments applied to dormant shoots from 27 March to 17 April resulted in an advancement of flowering by approximate 35 to 43 days (Table 1). For the application dates later than 30 April, the advancement of harvests was significantly reduced to between 17 to 20 days. Advancement of flowering for all BA-treated shoots in the greenpoint stage was between 41 and 51 days compared to that of the commercially harvested shoots. In addition, flowering time of shoots that received BA treatment on greenpoint buds later in the season (from 24 April up to 13 May) was more advanced than that of dormant-treated buds treated on the same dates (Table 1).

Table 2 represents the combined harvest distribution of both dormant and greenpoint treated shoots. Approximately 20% of commercially harvested stems were collected during week 11, around 13 March 2009. The harvest of the stems treated from 27 March to 24 April was focussed around week 6-7, resulting in an advancement of 32 to 42 days compared to the commercially harvested stems (Table 2). Shoots from the later remaining treatment dates displayed more widely-spread harvest peaks around week 7-10 (Table 2).

The commercial production stems harvested prior to 6 February contributed less than 5% of the total commercial harvest. This portion of the harvest is significantly lower than the percentage stems collected for all greenpoint and dormant BA-treated shoots from treatment dates 27 March to 17 April (Fig. 5). For these treatment dates 35-68% of BA-treated shoots were harvested before 6 February, but with no significant differences between the dormant and greenpoint treated shoots within treatment dates up to 17 April. For the later treatment dates from 24 April to 13 May, significantly more greenpoint treated shoots were harvested before week 6 when compared to dormant-treated shoots of the same treatment dates (Fig. 5).

The harvest date of stems bearing autumn-initiated inflorescences on 5-flush shoots in comparison to that of flowers that initiated on a six-flush shoot in spring were, however, only approximately between 1-2 weeks earlier and only so for the first three treatment dates, in dormant-treated shoots (Table 3). For the later treatment dates, flowers that initiated on six-flush shoots in spring was harvested in a comparable window or even earlier than flowers that initiated on the autumn flush (Table 3). Greenpoint-treated shoots that initiated inflorescences in autumn were harvested two to four weeks prior to six-flush shoots bearing spring-initiated inflorescences, when treated from 27 March to 10 April. Harvest dates for autumn-initiated inflorescences that were induced on greenpoint treated shoots on the later treatment dates were comparable to that of six-flush shoots. Significantly earlier harvests were recorded for six-flush spring-initiated shoots compared to the five-flush spring-initiated shoots, in both dormant and greenpoint treated shoots (Table 3).

INFLORESCENCE INITIATION PERCENTAGES ACCORDING TO RESPONSE TYPES. Inflorescences that initiated on five-flush shoots in spring were only represented in the case where dormant shoots were treated with BA, but did not induce a vegetative flush before winter (Table 3; Fig. 2). An average of 48% of all dormant-treated 'Pink Ice' shoots initiated an inflorescence in spring on a five-flush shoot according to its natural phenology (Table 3).

Flowering percentages of autumn-initiated inflorescences as recorded in control and dormant-treated shoots did not differ significantly, with 34% of untreated control shoots that initiated an inflorescence after producing a flush, before winter, compared to the 43% of treated dormant shoots that received treatment a MaxCel^{TM} application on the same date the control shoots were selected (Fig. 3B).

A low mean value of approximately 24 and 29% calculated over all treatment dates, for dormant BA-treated shoots were found to initiate inflorescences on a five-flush shoot in autumn or on a six-flush shoot in spring, respectively (Table 3). However, the first two treatment dates were consistently more successful at 41-44%, in initiating inflorescences in autumn on dormant shoots compared to later treatment dates. The propensity of dormant shoots treated from mid-April onwards to initiate autumn-induced inflorescences varied considerably, but was consistently lower than earlier treatment at 15.6-36.2% flower initiation over the treatment dates.

Generally, higher incidences of inflorescence initiation of between 39.6-73% were observed for inflorescences that initiated on a six-flush shoot in spring,

following BA treatment on dormant shoots in autumn (Table 3). However, these inflorescences were only prevalent on dormant shoots treated in March to the first week in May, excluding 30 April, with no recordings in this category for treatment dates on or later than 13 May (Table 3).

The percentage flowering recorded for the five-flush spring-initiated inflorescences was generally low for treatment dates in March and April (Table 3), but rapidly increased, with the lack of responsiveness of shoots to BA treatment in terms of budbreak with the later treatment dates of 30 April to 2 June (Fig. 4).

Inflorescence initiation in shoots treated at the greenpoint stage was only prevalent in one of two categories namely five-flush autumn-initiated and six-flush spring-initiated inflorescences. More inflorescences initiated in spring than in autumn for greenpoint-treated shoots at 56.2% compared to 42.7% flowering incidences in these categories, respectively (Table 3). Only treatment dates in April resulted in autumn-initiation rates of above 40%, while later treatment dates favoured inflorescence initiation on six-flush shoots in spring.

VEGETATIVE PHENOLOGY INFLUENCING FLOWERING TIME. When flowering was expressed as a percentage of the shoots that flushed in response to the BA-treatment applied during early autumn, comparative flowering incidences to that of untreated shoots was recorded (Fig. 6). BA application on greenpoint shoots from the second treatment date in April until the end of April resulted in higher flowering percentages than in dormant shoots, but did not differ significantly from that of the untreated shoots.

Dormant shoots treated with benzyladenine resulted in budbreak approximately three to four months earlier than the spring budbreak date, which was synchronized for all shoots to occur by the third week in August (Table 3). Dormant treated shoots on 27 March and 3-17 April were found to require significantly fewer days from application to budbreak than the later treatment dates, except for the greenpoint-treated shoots where budbreak was already in the naturally induced state on application (Table 3). The days from budbreak to harvest for greenpoint-treated shoots, where no lag phase between application and budbreak was experienced, were however, within the same range than for dormant-treated shoots with development periods on average 272±17 and 290±14 days respectively (Table 3).

When comparing the days required from budbreak to harvest for six-flush spring-initiated inflorescences to that of autumn-initiated inflorescences, a

significantly shorter development period was required for the spring-initiated inflorescences at 180±18 and 185±15 days for dormant and greenpoint treated shoots respectively (Table 3).

A significant, negative linear correlation (R^2=0.9659; *P*<.0001) was recorded between the date of budbreak and the number of days from budbreak to harvest for autumn-initiated inflorescences where early budbreak dates required the most days from budbreak to harvest (Fig. 7). Six-flush shoots that initiated inflorescences in spring required the least number of days from budbreak to harvest compared to either autumn-initiated shoots or where inflorescences were initiated in spring on five-flush shoots. These six-flush initiated-inflorescences completed the development of the subtending flush as well as that of the inflorescence within 180±18 and 185±15 days for dormant and greenpoint treated shoots, respectively (Fig. 7). Autumn-initiated inflorescences required between 244 and 292 days to complete their phenological development from budbreak to harvest for dormant-treated shoots and between 280 to 305 days for greenpoint-treated shoots, respectively. By comparison, spring-initiated inflorescences on five-flush shoots were able to complete their inflorescence development within a much shorter time span at an average 215±15 days from budbreak (Table 3; Fig. 7).

Regardless of the longer period required for five-flush, autumn-initiated shoot elongation and inflorescence development, these inflorescences were still harvested earlier compared to that of the five-flush spring-initiated inflorescences (Fig. 8). Similarly, inflorescences that initiated on six-flush shoots in spring, for both dormant and greenpoint-treated shoots, were also harvested later than five-flush autumn-initiated shoots, despite a much shorter development time required for spring-initiated inflorescences (Table 3; Fig. 8). This later harvest for inflorescences that initiated on six-flush shoots in spring can mainly be ascribed to a much later natural budbreak date in late August compared to the BA-induced budbreak ranging from April to end of June (Table 3; Fig. 8), as inflorescence development proceeded faster for spring-initiated inflorescences compared to that of autumn-initiated inflorescences (Fig. 9).

Inflorescences that initiated on five-flush shoots either in autumn or spring, developed according to a linear relationship, whilst a quadratic relationship was recorded for the development of inflorescences that initiated on six-flush shoots in spring (Fig. 9). This quadratic relationship associated with the development of the six-flush spring-initiated inflorescences implicate that a faster development rate was

achieved in these inflorescences later in the season than for the other two inflorescence types.

SHOOT AND INFLORESCENCE CHARACTERISTICS. Shoots treated at the greenpoint stage and which then initiated an inflorescence in autumn had superior vegetative characteristics in terms of stem diameter, leaf area and dry mass per leaf compared to any category of dormant-treated shoots (Table 4). Similar, greenpoint treated shoots that later produced an inflorescence on a six-flush shoot also had a greater leaf area and dry mass per leaf compared to that of dormant treated stems that produced flowers on six-flush shoots in spring. Outcomes of stem diameter, leaf areas and dry mass as collected at the time of treatment from dormant and greenpoint shoots, but later sorted according to the three respective response types, did not vary within these groups (Table 4). Dry mass per leaf, however, declined progressively from stems destined to carry autumn-induced inflorescences through to stems from which spring-initiated inflorescences were harvested (Table 4).

Stem diameter of shoots at harvest declined progressively from early to later treatment dates, so that thinner stems were generally associated with later treatment dates (Table 5). The number of leaves varied significantly between treatment dates, but did not display any specific trend over treatment dates (Table 5). No significant difference was observed in the total leaf area, leaf or total dry mass values for the shoots harvested from the respective treatment dates (Table 5).

Inflorescence characteristics differed considerably, but inconsistently between the respective treatment dates (Table 6). Inflorescences that initiated on April-treated stems were in general more extended (longer) than inflorescences from later treatment dates. However, no significant differences were noted for basal diameter or number of involucral bracts for inflorescences resulting from any treatment date (Table 6). For inflorescences associated with the treatment dates in May a lower number of florets, lighter colour and a lower total inflorescences dry weight were, in general, recorded compared to the earlier treatment dates (Table 6).

Discussion

BUDBREAK AND FLORAL INITIATION. *Protea* cv. 'Pink Ice' initiates inflorescences predominantly on the spring flush of over-wintering shoots, after a period of dormancy whilst flowers reach harvest maturity during March-May (Nieuwoudt, 2006). Floral initiation on an autumn flush of *Protea* cv. Sylvia (Gerber et al. 2001a) and 'Carnival' (Hoffman, 2006) permitted flowers to develop during winter and throughout spring to reach harvest maturity 6-8 weeks earlier than flowers which initiated on the spring flush according to standard phenological trends. In contrast to 'Sylvia' and 'Carnival' where mostly three-flush shoots were present on plants by early autumn (Gerber et al., 2001b; Hoffman, 2006), four-flush shoots dominated in the case of 'Pink Ice' when shoot growth was synchronised by pruning in late winter (Nieuwoudt and Jacobs, 2010). Since floral initiation occurs soon after bud sprouting during the early stages of shoot elongation (Gerber et al., 2001b; Hoffman et al., 2009) it is imperative that bud sprouting should occur in autumn to produce an autumn flush that may or may not initiate an out-of-season flower within that season.

Many (70%) four-flush shoots of 'Pink Ice' produced an autumn flush unassisted, but sprouting increased significantly to 96% when treated with benzyladenine (Fig. 3). This observation confirms that the application of benzyladenine indeed stimulates the incidence of budbreak in autumn in dormant *Protea* 'Pink Ice' shoots, similar to that reported for 'Carnival' (Hoffman et al., 2009). Flowering, expressed as a percentage of shoots that sprouted in autumn, was within the same range of approximately 38% for both BA-treated and untreated control shoots (Table 3; Fig. 6). Still, 35 shoots flowered per 100 shoots treated with BA which was more than the 28 achieved for the untreated control shoots (Fig. 3). This can most likely be ascribed to the higher percentage buds sprouted for BA-treated shoots compared to the untreated shoots (Fig. 3).

The period of natural bud sprouting in autumn lasted approximately three weeks as all sprouting of marked experimental shoots was completed by 17 April, by which time approximately 70% of shoots had sprouted (Fig. 3). After this date only the odd four-flush shoots sprouted unaided and although useful for experimental purposes, have no commercial significance.

More than 90% of dormant four-flush shoots treated with BA between 27 March and 10 April sprouted (Fig. 3-4). Thereafter, the percentage shoots sprouting decreased rapidly when BA application was delayed to between 17 April and 13 May,

with only 16% of shoots that sprouted when treated on the latter date. No budbreak could be induced on any shoots treated later in autumn, from 22 May to 2 June (Fig. 4). The reason for the onset and increase in intensity of bud dormancy is, however, unknown. It is unlikely that ecodormancy is the cause as buds sprouted readily in high percentages during early spring in August when temperatures are comparable to or lower than in the autumn month of May. If dormancy is of a correlative nature (paradormancy) the decline in sprouting is not due to a lack of cytokinin, as BA application in May to dormant terminal buds was ineffective to induce budbreak (Fig. 4). A similar lack of budbreak with BA application in late autumn was also reported in two-flush shoots, but not three-flush shoots of 'Carnival' (Hoffman, 2006).

As stated earlier, BA treatment of dormant shoots on 27 March did not increase floral initiation significantly compared to the untreated control (Fig. 3). Floral initiation expressed as a percentage of the shoots that sprouted varied from 22 to 45% for shoots treated with BA between 27 March and 24 April compared to 38% observed in control shoots (Fig. 6). The relatively high percentage flowering recorded in untreated control shoots again indicates that BA did not increase inflorescence initiation as was reported for 'Carnival' (Hoffman, 2006; Hoffman et al., 2009). Increasing the number of shoots that sprout and thereby providing more shoots an opportunity to initiate an inflorescence on this additional flush appears to be the main effect of BA in 'Pink Ice' in this study. More support for the latter deduction is obtained by the results where only the odd shoot sprouted unaided after 17 April, but 74% shoots sprouted when treated with BA on 24 April (Fig. 4) with a floral initiation percentage of 35% (Fig. 6). It is, therefore, concluded that BA at 500mg.L^{-1} induces bud sprouting, but not floral initiation in 'Pink Ice'. Concentrations of BA higher than 500mg.L^{-1} may induce more shoots to flower and should be evaluated. The relatively high incidence of floral initiation on dormant shoots treated late with BA could probably be due to the much closer proximity of induced budbreak dates to that of natural budbreak date, where shoots were most likely already in a physiological state associated with floral initiation that occurs naturally on the spring flush (Table 3).

It was shown that for 'Carnival' the propensity to initiate an inflorescence with BA treatment on the autumn flush decreased when the flush was immature (Hoffman, 2006). This phenomenon has also been reported for mango and citrus (Davenport, 2003). Shoot maturity in 'Pink Ice' may also explain the increase in autumn-induced

flowering percentages with BA application for shoots that reached green point unaided from 27 March to 10 April (Table 3; Fig. 5). Since 'Pink Ice' shoots flush more readily than 'Carnival' or 'Sylvia' the maturity state of the terminal flush varied considerable depending on the phase of flushing at the time of treatment. This variation in maturity is reflected in the shoot characteristics recorded at the time of treatment which almost showed a cyclic trend between treatments dates for the various reaction types (Table 4).

Synchronisation of shoot growth by pruning shoots in late winter is considered critical for 'Sylvia' and 'Carnival' to achieve out of season flower initiation, is also regarded to be of prime importance for 'Pink Ice' in order to achieve initiation of flowers on the autumn flush. Although more than 70 percent of four-flush shoots sprouted unaided in this experiment it is advisable that at the onset of the autumn flush, all four-flush shoots in the dormant or greenpoint phenological stage be treated with BA to maximise the potential number of shoots that can sprout in autumn. This may assist to improve synchronisation of sprouting and thus maximise the number of shoots that may flower on an autumn flush, cognisant of the finding from this study that BA by itself does not increase floral initiation in 'Pink Ice'.

TIME OF FLOWERING. The data revealed that the relationship between the date of greenpoint and inflorescence initiation can be separated into three different response reaction groups (Fig. 2) namely: Shoots that sprouted in autumn followed by development of an inflorescence (five-flush autumn-initiated inflorescence); shoots that sprouted autumn and again in spring where after an inflorescence initiated (six-flush spring-initiated inflorescence); and shoots that failed to sprout in autumn, but did so in spring, followed by flowering on a spring-flush (five-flush spring-initiated inflorescence). The time of flowering for inflorescences that initiated on an autumn flush was governed, firstly, by the date of budbreak (greenpoint) and then, secondly, by the period from greenpoint to harvest maturity. For flowers that initiated on an autumn flush, the linear decrease in the number of days from greenpoint to harvest maturity with later dates of budbreak (Fig. 2) is possibly temperature related. Flowers that initiated in April included the entire winter in the floral development period, whereas flowers that initiated in mid-winter only included the latter part of winter. The generally higher temperatures known to be present after winter resulted in a shorter development period to harvest for late-initiated flowers. Despite the longer development period for flowers that initiated early in autumn, the flowering time was

advanced in a linear trend where earlier greenpoint was achieved in autumn (Fig. 9). The time that was gained by earlier induced-greenpoint more than offset the time lost by a longer development period for these flowers. To achieve early flowering it is therefore important that the autumn flush of shoot growth should attain greenpoint from early April to mid May (Fig. 8). If greenpoint is attained after mid May, the flowering time is comparable to flowers that initiated on a six-flush shoot in spring (Fig. 8).

The number of flushes that subtends a spring-initiated flower has a profound effect on the time when harvest maturity was attained (Table 3). The period of natural bud sprouting in spring is synchronized, short and is completed within a week to a 14-day period in August. Increasing the size of the source from five to six flushes reduced the period from greenpoint to harvest on average by 35 days (Table 3), and advanced the harvest date on average by approximately 23 days, possibly due to the greater source activity. The flowering time of five-flush shoots that sprouted and initiated flowers on an autumn flush, on or before 24 April, was advanced by one to four weeks and by an additional three weeks compared to six-flush and five-flush shoots, respectively, that initiated flowers in spring.

Greenpoint-treated shoots in general showed a greater advancement in harvest time relative to the control than that of dormant shoots, especially for later treatment dates (Table 1). Dormant shoots treated with MaxCel^{TM} had the distinct disadvantage compared to greenpoint shoots in that the days to budbreak, which was already achieved in greenpoint shoots, increased significantly as autumn progressed into the cooler months of late April and May, to such an extent that no budbreak could be achieved beyond 22 May (Fig. 4). Later harvests in dormant-treated shoots compared to that of greenpoint- treated shoots not only reflected a later budbreak date of dormant-treated shoots, but also the inferior shoot quality generally recorded in dormant shoots compared to that greenpoint shoots at the time of treatment. This was true for shoot diameter, leaf area and leaf dry mass in shoots that would initiate autumn inflorescences, but was also the case for area per leaf and leaf dry mass in shoots that would later initiate an inflorescence on a six-flush shoot in spring (Table 4).

When the harvest for the commercially produced stems was compared to that of BA-treated shoots, irrespective of the phenological stage of the terminal bud, the commercial production peaked more than a month later than those treated in March

through to middle May (Table 2). Furthermore, a significant number of dormant and greenpoint shoots treated in April were harvested just before 6 February, with up to 68% and 52% of stems for the first April application, respectively, compared to the >5% of commercial harvest up to the same date (Fig. 5). The wide-spread harvest distribution of the commercially produced stems from late March to end June may be a result of a lack of synchronized flushing in a range of untreated shoots, exhibiting varying shoot characteristics (Table 2).

Product quality as defined by inflorescence and shoot characteristics at harvest was compared over treatment dates. Shoots treated later in the season were significantly thinner, with generally a lower number of leaves at harvest than shoots treated earlier in the season (Table 5). Furthermore, when considering inflorescence quality at harvest, treatment times resulted in significant differences in the length of the inflorescence, the number of florets, the total dry weight as well as the colour (Table 6). These differences are not unexpected as inflorescences that were treated later in the season developed under different environmental conditions to those initiated earlier in the season. However, all harvested stems, irrespective of treatment time or phenological stage treated, were of marketable quality (personal observation).

Conclusion

Treating four-flush shoots of *Protea* 'Pink Ice' at the onset of autumn with BA secured the production of an additional flush before winter. This more or less eliminated the occurrence of five-flush shoots that initiate flowers in spring. Some 30 to 40 per cent of the shoots should initiate a flower on the autumn flush which then should reach harvest maturity between the first week of January and mid-February. Shoots that do not initiate a flower in autumn on the induced flush will do so in spring on a six-flush shoot that will flower after the first week and up to the end of February.

From a marketing view point it would be desirable if the flowering time could be advanced to November and December. Earlier work by Nieuwoudt (2006) showed that this can be achieved if regrowth on bearers started in March with the result that before spring one or two flush shoots were present on the plant. Thus in May the following year, after completion of the autumn flush, a flower subtended by a six- or seven-flush shoot will be initiated that may be harvested by December. Some of the shoots will, however, initiate a flower on short, over-wintering shoots in spring, following pruning in March earlier the same year. Removal of these flowering shoots

is recommended as it may interfere with the floral initiation on the autumn flush as was shown to be the case for 'Carnival' (Hoffman and Jacobs, 2012). Such an additional management intervention may allow for higher floral initiation earlier in autumn, than could be achieved in this study. Without additional sinks as competition for young, BA-induced developing inflorescence, an advanced flowering time within the required November to December marketing window may be achieved.

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Table 1. The advancement of flowering time and harvest distribution of *Protea* cv. Pink Ice flowering stems when terminal buds on four-flush shoots were treated weekly either at greenpoint or in the dormant stage with MaxCel™ at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) in the autumn of 2008. Harvest distribution values are expressed as percentages of the total harvest. Shaded blocks represent the peak harvest dates where harvest percentages were equal to or greater than 15%. Stems were harvested at the commercial 'soft tip' stage from 28 November 2008 to 29 June 2009.

					DATE OF HARVEST														
					Harvest weeks of 2008-2009														
	Mean harvest date of harvested stems	STDev of mean harvest date (±days)	Harvest adv. (days)[z]	Number of stems	48-51	1	2	3	4	5	6	7	8	9	10	11	12	13	14-29
					28-Nov - 19-Jan	02-Jan	09-Jan	16-Jan	23-Jan	30-Jan	06-Feb	13-Feb	20-Feb	27-Feb	06-Mar	13-Mar	20-Mar	27-Mar	30-Mar – 29-Jun
Commercial production	23-Mar	37		32332	0.23	0.28	0.08	0.23	0.52	0.70	1.55	1.88	1.74	3.39	8.52	19.95	13.68	9.65	37.60
Dormant shoots																			
27-Mar	11-Feb	16	-40	43		2.33		4.65	2.33	16.28	27.91	18.60	9.30	6.98	4.65	2.33		4.65	
03-Apr	8-Feb	13	-43	47			2.13	2.13	4.26	14.89	44.68	17.02	8.51		2.13	2.13			2.13
10-Apr	10-Feb	11	-41	47				4.26		12.77	31.91	31.91	12.77	4.26				2.13	
17-Apr	15-Feb	8	-35	41							26.83	26.83	31.71	7.32	7.32				
24-Apr	22-Feb	10	-29	48							8.33	14.58	39.58	25.00	8.33			2.08	2.08
30-Apr	5-Mar	12	-18	49								2.04	16.33	30.61	22.45	18.37		6.12	4.08
08-May	2-Mar	13	-20	47				2.13	2.13			2.13	8.51	29.79	36.17	8.51	2.13	6.38	2.13
13-May	4-Mar	11	-19	45					2.22				11.11	28.89	31.11	20.00	2.22	2.22	2.22
22-May	4-Mar	8	-18	47							2.13		4.26	29.79	36.17	23.40	2.13	2.13	
02-Jun	5-Mar	7	-17	47							2.13		2.13	23.40	40.43	29.79	2.13		
Greenpoint shoots																			
27-Mar	16-Feb	19	-41	44	2.27	2.27			9.09	4.55	13.64	18.18	4.55	27.27	4.55	9.09		4.55	
03-Apr	11-Feb	18	-47	46			4.35	4.35	8.70	15.22	19.57	15.22	8.70	6.52	8.70	4.35	2.17	2.17	
10-Apr	07-Feb	13	-51	48		4.17	2.08	4.17	2.08	14.58	25.00	31.25	8.33	6.25	2.08				
17-Apr	10-Feb	12	-47	46				4.35	4.35	8.70	32.61	19.57	19.57	6.52	2.17	2.17			
24-Apr	15-Feb	13	-43	48					6.25	6.25	18.75	27.08	20.83	12.50	2.08	4.17			2.08
30-Apr	15-Feb	9	-43	46						2.17	19.57	47.83	15.22	10.87		2.17	2.17		
08-May	15-Feb	8	-43	31							19.35	48.39	22.58	6.45			3.23		
13-May	12-Feb	27	-46	22	9.09			4.55			9.09	9.09	36.36	18.18	9.09	4.55			

[z] The advancement of the harvest date of treated shoots was calculated by subtracting the mean commercial peak from the mean harvest date of the treated shoots' production date.

Table 2. The harvest advancement and distribution of both dormant and greenpoint shoots combined, of *Protea* cv. Pink Ice inflorescences, following a treatment of MaxCel™ at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) when applied to the terminal bud of four-flush shoots, either in the dormant and greenpoint stage in the autumn of 2008. Values are expressed as percentages of the total harvest. Shaded blocks represent the peak harvest dates where harvest percentages were equal to or greater than 15%. Stems were harvested at the commercial "soft tip" stage from 28 November 2008 to 29 June 2009.

					DATE OF HARVEST													
					Harvest weeks of 2008-2009													
					48-51	1	2	3	4	5	6	7	8	9	10	11	12	13	14-29
	Mean harvest date of harvested stems	STDev (±days)	Harvest adv. (days)[z]	Number of stems	28-Nov - 19-Jan	02-Jan	09-Jan	16-Jan	23-Jan	30-Jan	06-Feb	13-Feb	20-Feb	27-Feb	06-Mar	13-Mar	20-Mar	27-Mar	30-Mar – 29-Jun
Commercial production	23-Mar	37		32332	0.23	0.28	0.08	0.23	0.52	0.70	1.55	1.88	1.74	3.39	8.52	19.95	13.68	9.65	37.60
Treatment date 2008																			
27-Mar	13-Feb	18	-37	87	1.15	2.30		2.30	5.75	10.34	20.69	18.39	6.90	17.24	4.60	5.75		4.60	
03-Apr	9-Feb	16	-41	93			3.23	3.23	6.45	15.05	32.26	16.13	8.60	3.23	5.38	3.23	1.08	1.08	1.08
10-Apr	8-Feb	12	-42	95		2.11	1.05	4.21	1.05	13.68	28.42	31.58	10.53	5.26	1.05			1.05	
17-Apr	13-Feb	10	-38	87				2.30	2.30	4.60	29.89	22.99	25.29	6.90	4.60	1.15			
24-Apr	18-Feb	12	-32	96					3.13	3.13	13.54	20.83	30.21	18.75	5.21	2.08		1.04	2.08
30-Apr	24-Feb	14	-27	95						1.05	9.47	24.21	15.79	21.05	11.58	10.53	1.05	3.16	2.11
08-May	24-Feb	13	-26	78				1.28	1.28		7.69	20.51	14.10	20.51	21.79	5.13	2.56	3.85	1.28
13-May	25-Feb	20	-25	67	2.99			1.49	1.49		2.99	2.99	19.40	25.37	23.88	14.93	1.49	1.49	1.49
22-May	4-Mar	8	-18	47							2.13	0.00	4.26	29.79	36.17	23.40	2.13	2.13	
02-Jun	5-Mar	7	-17	47							2.13	0.00	2.13	23.40	40.43	29.79	2.13		

[z] The advancement of the harvest date of treated shoots was calculated by subtracting the mean commercial peak from the mean harvest date of the treated shoots production date.

Table 3. The advancement of flowering time, the harvest date (in Julian days) and the percentage (%) flowering of five-flush autumn-initiated inflorescences as well as that of five- and six-flush spring-initiated flowering stems of four-flush *Protea* cv Pink Ice shoots treated with MaxCel™ in comparison to a commercial production system. Stems were treated terminally either in the dormant or the greenpoint stage with a MaxCel™ solution at 500 mgL^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) on various dates during the autumn of 2008. The date of budbreak and days from budbreak to harvest is also presented for the various reaction responses (five-flush autumn-, six-flush spring- and five-flush spring-initiated) of four-flush 'Pink Ice' shoots to the MaxCel™ application.

		AUTUMN-FLUSH INITIATED INFLORESCENCES							SPRING-FLUSH INITIATED INFLORESCENCES								
		Five-flush shoots							Six-flush shoots					Five-flush shoots			
Treatment stage and date (2008)	Total number of flowers harvested (2008-9)	Date of budbreak ± STDev (days)	Days from budbreak to harvest ±STDev (days)	Days to bud-break (from trt)	Δ6SFl	Δ5SFl	Harvest date ± STDev (Julian day)	Flowering (%) of total stems harvested	Date of budbreak ± STDev (days)	Days of budbreak to harvest ±STDev (days)	Days to bud-break (from trt)	Harvest date ± STDev (Julian day)	Flowering (%) of total stems harvested	Date of budbreak ± STDev (days)	Days from budbreak to harvest ±STDev (days)	Days to bud-break (from trt)	Harvest date ± STDev (Julian day)	Flowering (%) of total stems harvested
Dormant																		
27-Mar	43	12-Apr±4	292±11	17	18	52	30±11	41.9	15-Aug±0	186±10	141	48±10	53.5	24-Aug±13	211±13	151	82±0	4.7
03-Apr	47	18-Apr±6	289±9	16	7	44	33±9	44.7	17-Aug±7	176±10	136	40±7	48.9	21-Aug±11	208±5	140	77±15	6.4
10-Apr	47	21-Apr±6	289±11	11	7	25	36±10	31.9	22-Aug±16	173±20	135	43±7	63.8	15-Aug±0	199±34	127	61±34	4.3
17-Apr	41	9-May±24	284±19	22	-3	17	48±11	22	27-Aug±9	171±10	133	45±6	73.2	15-Aug±0	203±0	120	65±0	4.9
24-Apr	48	7-Jun±28	260±27	44	-6	7	54±5	35.4	16-Aug±23	184±27	115	48±8	39.6	30-Jul±16	215±21	97	61±14	25
30-Apr	49	28-Jun±33	244±37	59	*	7	58±6	8.2	*	*	*	*	0	4-Aug±19	213±20	96	65±12	91.8
08-May	47	16-Jun±4	258±13	39	-8	5	60±12	36.2	15-Aug±0	190±30	99	50±30	8.5	24-Jul±14	224±17	78	65±9	55.3
13-May	45	17-Jun±0	261±7	35	*	-1	64±7	15.6	*	*	*	*	0	21-Jul±13	225±19	70	63±11	84.4
22-May	47	*	*	*	*	*	*	0	*	*	*	*	0	21-Jul±10	227±13	61	65±7	100
02-Jun	47	*	*	*	*	*	*	0	*	*	*	*	0	25-Jul±9	223±12	53	65±7	100
Means		**20-May±13**	**272±17**	**30**			**48±9**	**23.6**	**19-Aug±9**	**180±18**	**126**	**44±9**	**28.8**	**4-Aug±11**	**215±15**	**99**	**67±11**	**47.6**
Greenpoint																		
27-Mar	44	27-Mar±0	302±15		33	*	23±15	25	14-Aug±3	194±13		56±13	75	*	*		*	0
03-Apr	46	3-Apr±0	305±16		16	*	33±16	43.5	15-Aug±0	187±16		49±16	56.5	*	*		*	0
10-Apr	48	10-Apr±0	297±13		14	*	32±13	58.3	16-Aug±6	182±10		46±9	41.7	*	*		*	0
17-Apr	46	17-Apr±0	297±12		7	*	39±12	63	17-Aug±6	182±10		46±10	37	*	*		*	0
24-Apr	48	24-Apr±0	290±9		16	*	39±9	54.2	19-Aug±8	189±17		55±13	45.8	*	*		*	0
30-Apr	46	30-Apr±0	286±5		8	*	41±5	34.8	19-Aug±24	182±23		49±10	65.2	*	*		*	0
08-May	31	8-May±0	280±5		5	*	43±5	35.5	19-Aug±8	181±12		48±9	64.5	*	*		*	0
13-May	22	13-May±0	282±10		2	*	29±39	36.4	24-Aug±12	180±20		51±13	63.6	*	*		*	0
Means		**20-Apr±0**	**292±14**				**35±11**	**43.8**	**18-Aug±8**	**185±15**		**50±12**	**56.2**	*	*		*	0

Δ6Fl & Δ5Fl: Advancement (days) of flowering time of the autumn-initiated inflorescences compared to the flowering time of the six-flush spring-initiated or five-flush spring-initiated inflorescences respectively.
*No response observed.

Table 4. Shoot characteristics of *Protea* cv. 'Pink Ice' at the time of treatment with MaxCel™ at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) in the autumn of 2008. Shoots were divided into three response types that resulted from the treatment at either the greenpoint or dormant phenological stage: shoots with inflorescence initiation in autumn on five-flushes; shoots with BA-induced flush formation in autumn, but with inflorescence initiation on six-flushes in spring and inflorescence initiation on five-flush shoots in spring, without a BA-induced flush in autumn.

	SHOOT CHARACTERISTICS AT TIME OF TREATMENT											
	AUTUMN-FLUSH INITIATED INFLORESCENCES				SPRING-FLUSH INITIATED INFLORESCENCES							
	Five-flush shoots				Six-flush shoots				Five-flush shoots			
Treatment date 2008	Number of stems	Intercalation diameter of treated flush ±SE (mm)	Area per leaf ±SE (cm^2)	Dry mass per leaf ±SE (g)	Number of stems	Intercalation diameter of treated flush ±SE (mm)	Area per leaf ±SE (cm^2)	Dry mass per leaf ±SE (g)	Number of stems	Intercalation diameter of treated flush ±SE (mm)	Area per leaf ±SE (cm^2)	Dry mass per leaf ±SE (g)
Dormant												
27-Mar	18	8.00±0.13	15.06±0.00	0.27±0.01	26	7.60±0.12	14.25±0.00	0.25±0.11	2	6.72±0.28	11.85±0.00	0.18±0.00
03-Apr	22	7.53±0.13	13.62±0.00	0.22±0.01	24	7.11±0.11	12.98±0.00	0.20±0.15	3	7.48±0.39	14.90±0.00	0.25±0.01
10-Apr	16	7.66±0.13	14.26±0.00	0.23±0.02	30	7.43±0.10	14.00±0.00	0.22±0.12	2	7.61±0.47	11.28±0.00	0.16±0.01
17-Apr	11	7.15±0.14	11.63±0.00	0.18±0.02	31	7.06±0.08	13.21±0.00	0.21±0.11	2	7.25±0.31	10.39±0.00	0.14±0.00
24-Apr	18	7.46±0.11	16.16±0.00	0.30±0.01	19	7.67±0.16	16.09±0.00	0.31±0.07	12	7.55±0.91	15.80±0.00	0.30±0.01
30-Apr	4	7.34±0.35	15.01±0.00	0.28±0.03	*	*	*	*	46	7.49±0.09	14.78±0.00	0.27±0.01
08-May	17	7.49±0.13	15.34±0.00	0.29±0.01	4	7.63±0.50	13.66±0.00	0.25±0.03	28	7.62±0.10	14.84±0.00	0.29±0.01
13-May	8	7.80±0.23	14.01±0.00	0.28±0.01	*	*	*	*	42	7.84±0.07	14.82±0.00	0.29±0.01
22-May	*	*	*	*	*	*	*	*	49	7.73±0.07	15.96±0.00	0.32±0.01
02-Jun	*	*	*	*	*	*	*	*	47	7.84±0.06	15.55±0.00	0.31±0.01
Avg ± SE		**7.55±0.17**	**14.38±0.63**	**0.26±0.01**		**7.42±0.18**	**14.03±0.40**	**0.24±0.10**		**7.51±0.20**	**14.02±0.38**	**0.25±0.01**
Greenpoint												
27-Mar	11	7.84±0.18	15.57±0.00	0.30±0.01	35	7.55±0.09	15.51±0.00	0.32±0.07	*	*	*	*
03-Apr	20	7.44±0.09	15.95±0.00	0.27±0.01	28	7.25±0.11	15.47±0.00	0.28±0.07	*	*	*	*
10-Apr	28	7.76±0.11	15.50±0.00	0.26±0.01	21	7.27±0.09	15.55±0.00	0.28±0.10	*	*	*	*
17-Apr	29	7.57±0.11	17.18±0.00	0.32±0.01	18	7.29±0.10	15.07±0.00	0.27±0.10	*	*	*	*
24-Apr	27	7.64±0.08	15.38±0.00	0.29±0.01	22	7.36±0.09	14.95±0.00	0.29±0.09	*	*	*	*
30-Apr	17	7.77±0.12	15.50±0.00	0.29±0.02	33	7.55±0.10	15.45±0.00	0.29±0.11	*	*	*	*
08-May	13	8.09±0.12	17.07±0.00	0.31±0.01	22	7.54±0.13	15.69±0.00	0.29±0.09	*	*	*	*
13-May	6	8.08±0.34	15.92±0.00	0.29±0.01	14	7.65±0.18	16.27±0.00	0.30±0.05	*	*	*	*
Avg ± SE		**7.78±0.14**	**16.01±0.55**	**0.29±0.01**		**7.43±0.11**	**15.58±0.56**	**0.29±0.09**	*	*	*	*

*No response observed.

Table 5. Shoot characteristics subtending the inflorescence of *Protea* 'Pink Ice' when harvested at the 'soft tip' commercially-ready stage, after being treated with a MaxCel™ solution at 500 mg.L^{-1} (active ingredient: 6-benzyladenine 1.9%) on ten consecutive dates in the autumn of 2008.

	CHARACTERISTICS OF HARVESTED STEMS				
Treatment date (2008)	Stem diameter (mm)	Number of leaves	Total leaf area (cm^2)	Total leaf dry mass (g)	Total dry mass (g)
27-Mar	14.50±0.8	63±3	801.42±65.5	21.28±1.6	41.50±2.4
03-Apr	13.13±0.5	63±2	1057.06±201.5	22.81±1.3	42.78±2.5
10-Apr	13.36±0.7	50±5	768.14±52.8	19.50±1.3	38.21±1.6
17-Apr	13.32±0.3	56±4	856.09±84.3	21.80±2.0	41.86±3.0
24-Apr	13.44±0.3	48±3	624.12±39.8	17.00±0.8	35.92±3.0
30-Apr	13.15±0.7	60±4	966.40±61.0	23.82±1.6	43.38±2.9
08-May	12.54±0.4	54±2	815.27±40.26	19.95±0.7	37.73±1.3
13-May	12.61±0.4	52±6	768.28±68.6	19.38±1.8	37.71±1.3
22-May	12.16±0.2	57±2	758.95±24.2	19.37±0.8	36.18±2.1
02-Jun	11.64±0.4	64±2	822.93±44.0	20.45±1.2	36.84±2.3
P-value	0.023	0.017	0.060	0.054	0.396
F-value	2.50	2.63	2.04	2.09	1.08

Table 6. Inflorescence characteristics of *Protea* cv. Pink Ice at the commercial 'soft tip' stage when harvested throughout January 2009. The inflorescences initiated either on an autumn- or spring-flush when four-flush shoots received a terminal application of MaxCel™ at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) on ten consecutive treatment dates from March to June 2008 (n=5).

	INFLORESCENCE CHARACTERISTICS					
Treatment date (2008)	Length (mm)	Basal Diameter (mm)	Number of Involucral bracts	Number of Florets	Colour (Hue)	Total dry weight of inflorescence (g)
27-Mar	101.9±1.7^{cd}	54.3±1.9^{ab}	124.6±3.1^{abc}	373.4±7.1^{ab}	27.2±1.3^{abc}	31.4±2.5^{ab}
03-Apr	105.0±0.9^{abc}	56.2±1.0^{ab}	118.8±1.8^{bc}	361.6±4.5^{b}	26.8±1.2^{abc}	34.0±0.4^{a}
10-Apr	105.6±0.7^{a}	55.0±2.0^{ab}	128.0±4.5^{ab}	363.2±9.0^{b}	26.2±1.2^{abc}	32.5±1.2^{a}
17-Apr	105.2±1.1^{ab}	53.4±1.0^{b}	123.8±2.5^{abc}	360.4±3.6^{b}	27.7±1.3^{ab}	32.3±1.1^{a}
24-Apr	103.4±1.0^{abc}	56.8±1.1^{a}	128.8±3.0^{a}	392.0±10.4^{a}	27.1±1.0^{abc}	33.4±0.9^{a}
30-Apr	103.5±1.3^{abc}	56.1±0.7^{ab}	117.2±1.2^{c}	352.0±3.9^{bc}	24.4±1.1^{bcd}	31.8±1.2^{a}
08-May	102.3±0.3^{bcd}	55.3±0.9^{ab}	115.8±4.6^{c}	355.8±13.1^{bc}	26.9±1.7^{abc}	30.9±0.6^{abc}
13-May	101.2±1.2^{cd}	54.1±0.8^{ab}	121.4±5.0^{abc}	371.6±12.4^{ab}	29.0±1.3^{a}	31.0±0.8^{ab}
22-May	100.3±1.2^{d}	53.8±0.9^{ab}	123.0±4.7^{abc}	334.0±10.6^{c}	24.2±1.1^{cd}	28.1±1.2^{bc}
02-Jun	101.3±0.7^{cd}	54.5±1.1^{ab}	120.8±2.0^{abc}	350.0±6.7^{bc}	21.3±0.9^{d}	27.5±1.2^{c}
P-value	0.007	0.549	0.178	0.006	<0.001	0.010
F-value	3.09	0.88	1.51	3.15	3.34	2.90

Means with the same letter are not significantly different, LSD (p=0.05).

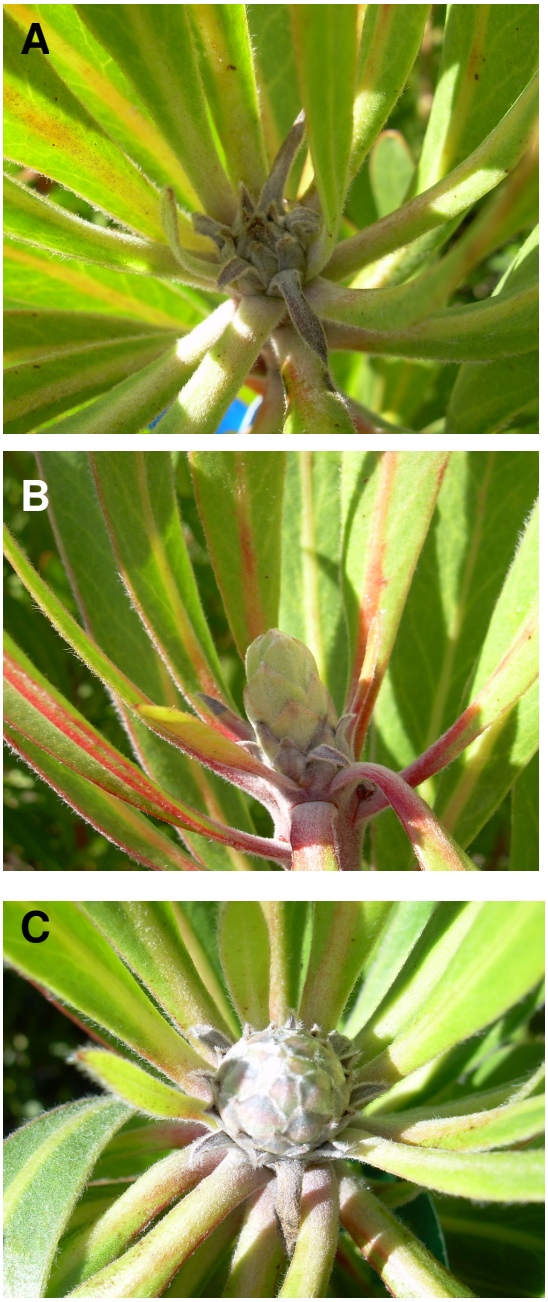


Fig. 1. The phenological growth stages of the terminal bud on a four-flush shoot of *Protea* cv. Pink Ice: (**A**) the dormant, terminal bud with no visible signs of active growth, where the bud scales are dry and contracted; (**B**) the 'budbreak' or 'greenpoint stage' signalling the appearance of the preformed leaves of a successive vegetative growth flush and where the terminal bud represent a compact torpedo-shaped structure of approximately 10 mm in length; (**C**) a young, developing inflorescence of approximately 10 mm in diameter.

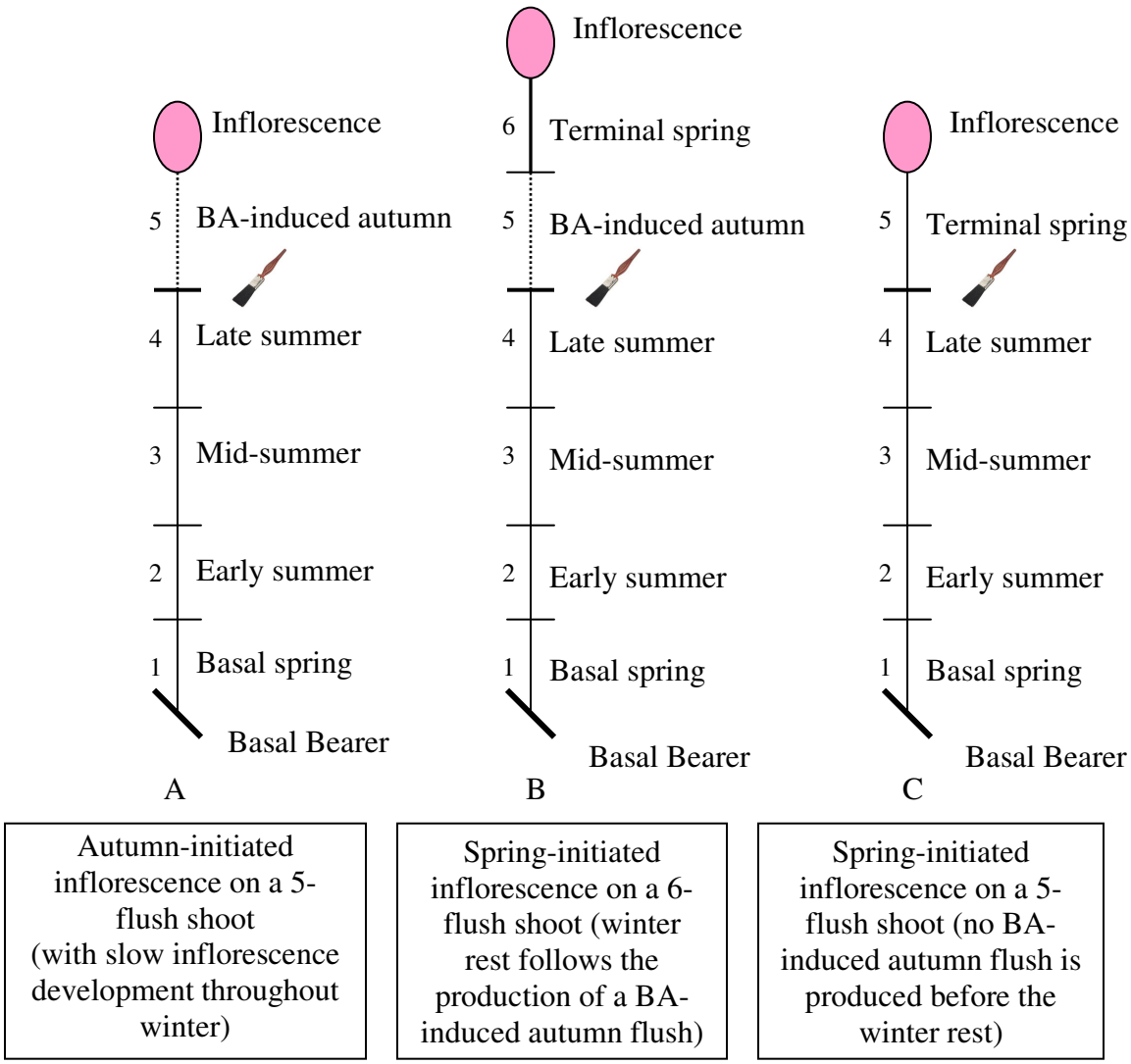
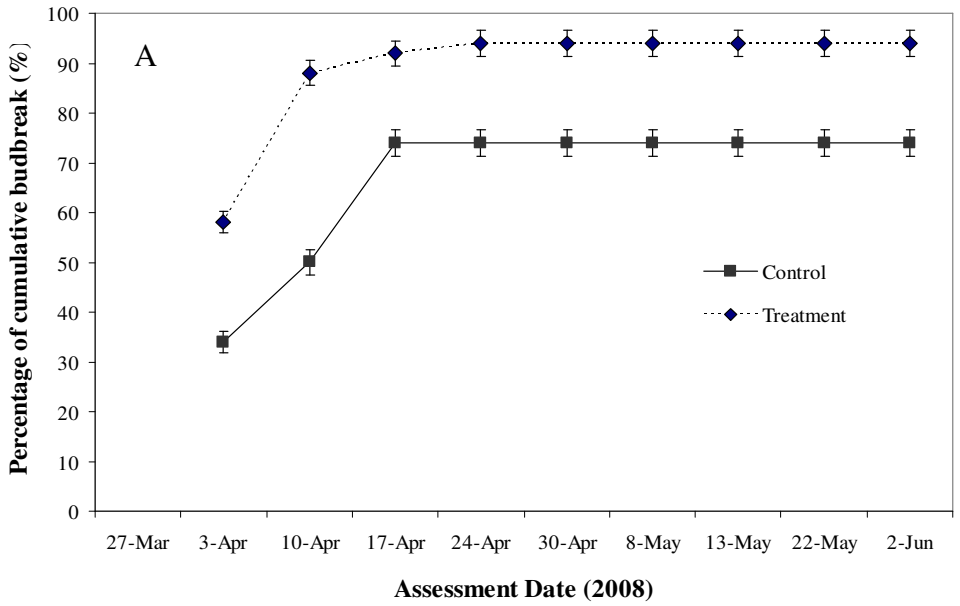
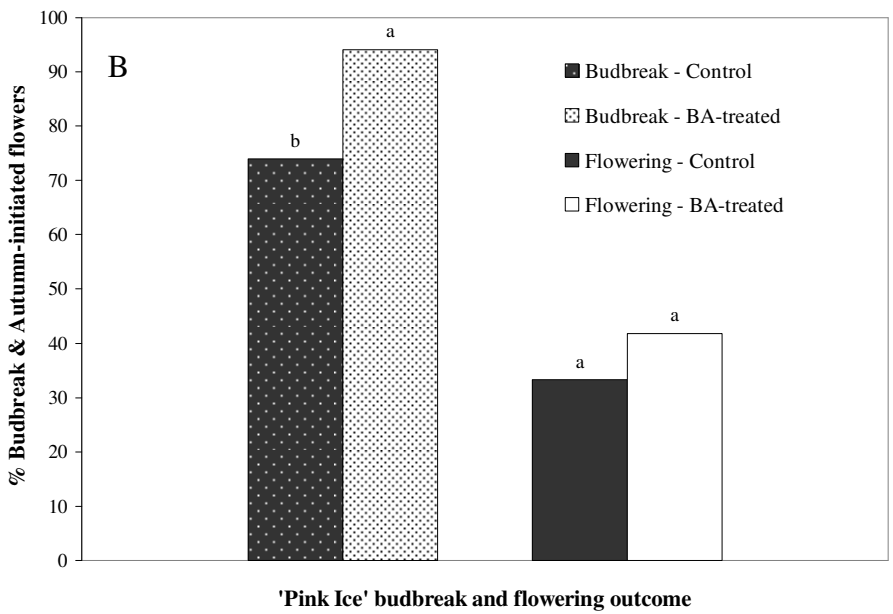


Fig. 2. The various responses of a four-flush shoot of *Protea* cv Pink Ice, consisting of a basal spring flush, a 1st (early) summer-, a 2nd (mid-) summer- and a 3rd (late-) summer flush, when treated terminally in autumn with Maxcel™ at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%). (**A**) The production of a BA-induced vegetative, autumn flush (--), shortly after treatment, but prior to winter, which is then shortly thereafter followed by inflorescence initiation and development; (**B**) the production of a BA-induced, vegetative autumn flush prior to winter, but with no inflorescence initiation on the induced flush prior to winter. The terminal buds remain vegetative, but dormant throughout winter. A synchronous spring flush is produced after a period of winter rest and terminates subsequently in an inflorescence, following directly after flush elongation; (**C**) no vegetative flush is induced in autumn in reaction to BA application and the terminal bud remains dormant throughout winter until spring, whereafter it follows the normal phenology of inflorescence initiation on a vigorous spring-flush, with anthesis in the following summer.





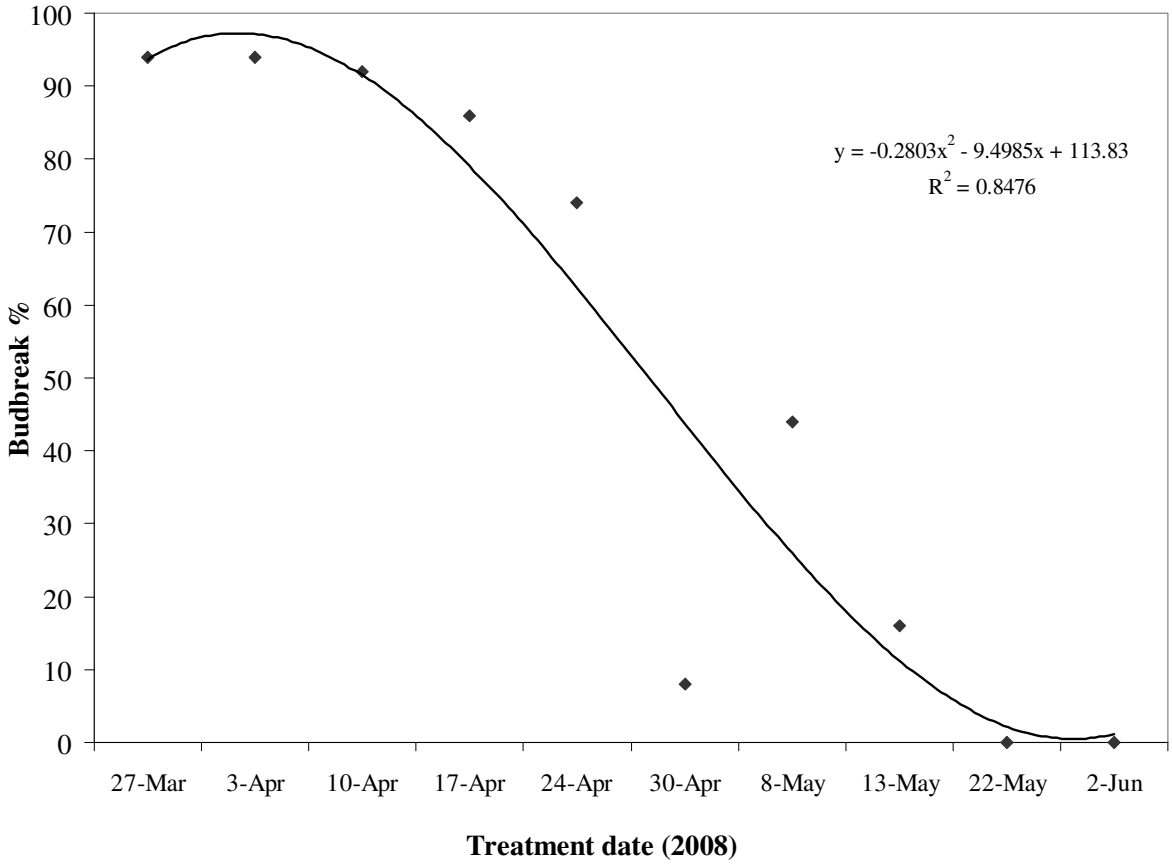
ANOVA

SOURCE	*F value*	*Pr > F*	*Significance*
Control	11.56	<.0001	***
Treatment	18.00	<.0001	***

ANOVA

SOURCE	*F value*	*Pr > F*	*Significance*
Budbreak	18.68	0.012	*
Flowering	0.02	0.893	ns

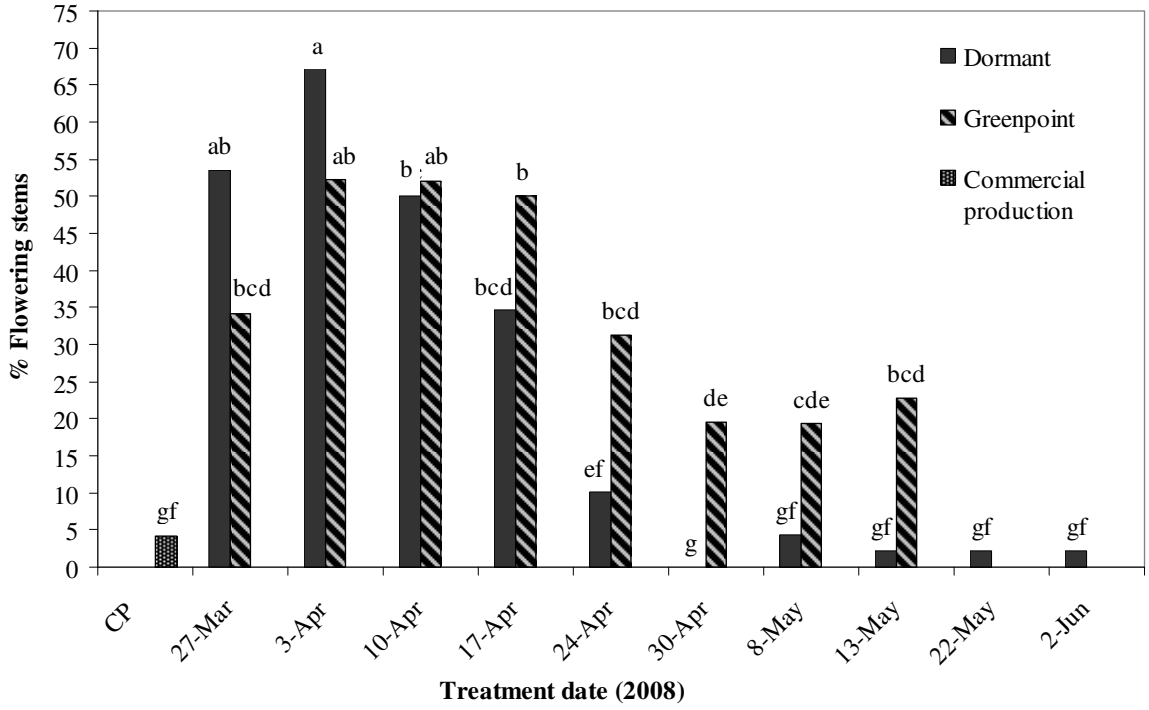
Fig. 3. (**A**) Percentage (%) cumulative autumn-induced budbreak when dormant four-flush *Protea* 'Pink Ice' shoots were treated with MaxCel^{TM} at 500 mgL^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) on 27 March 2008 compared to that of control, untreated shoots observed over the same period (n=50). (**B**) The percentage (%) of natural budbreak and natural autumn-induced inflorescence initiation on four-flush *Protea* 'Pink Ice' shoots, selected on 27 March 2008, compared to the budbreak (%) and autumn-induced inflorescence initiation of dormant shoots treated with MaxCel^{TM} at 500 mgL^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) on the same date (n=50). Means with the same letter are not significantly different, LSD (*P*=0.05).



ANOVA

SOURCE	*F value*	*Pr > F*	*Significance*
Dormant	21.00	<.0001	***
CONTRASTS			
Time Lin	94.21	<.0001	***
TimeQuad	7.75	0.0085	*

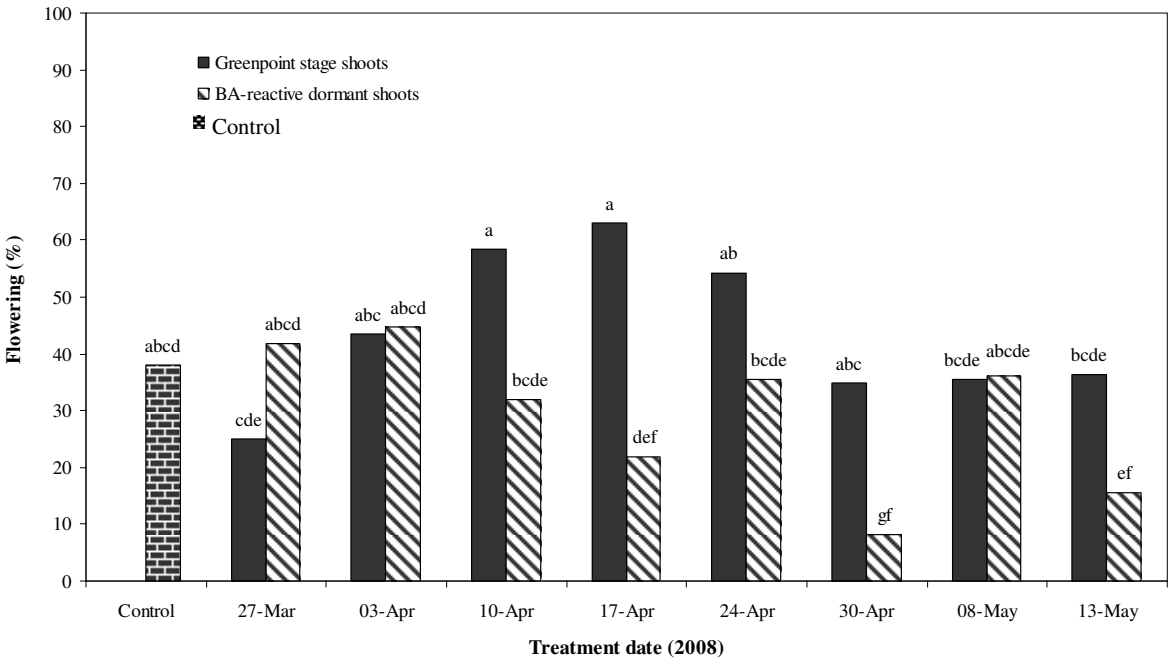
Fig. 4. The budbreak percentage (%) induced on four-flush, dormant *Protea* 'Pink Ice' shoots with application of MaxCel™ solution concentrations at 500 $mg.L^{-1}$ to the terminal bud (active ingredient: 6-benzyladenine (BA) 1.9%) on ten consecutive dates during the autumn of 2008 (n=50).



ANOVA

SOURCE	*F value*	*Pr > F*	*Significance*
Treatment	13.91	<.0001	***
Commercial production (CP) vs. treatment	24.57	<.0001	***

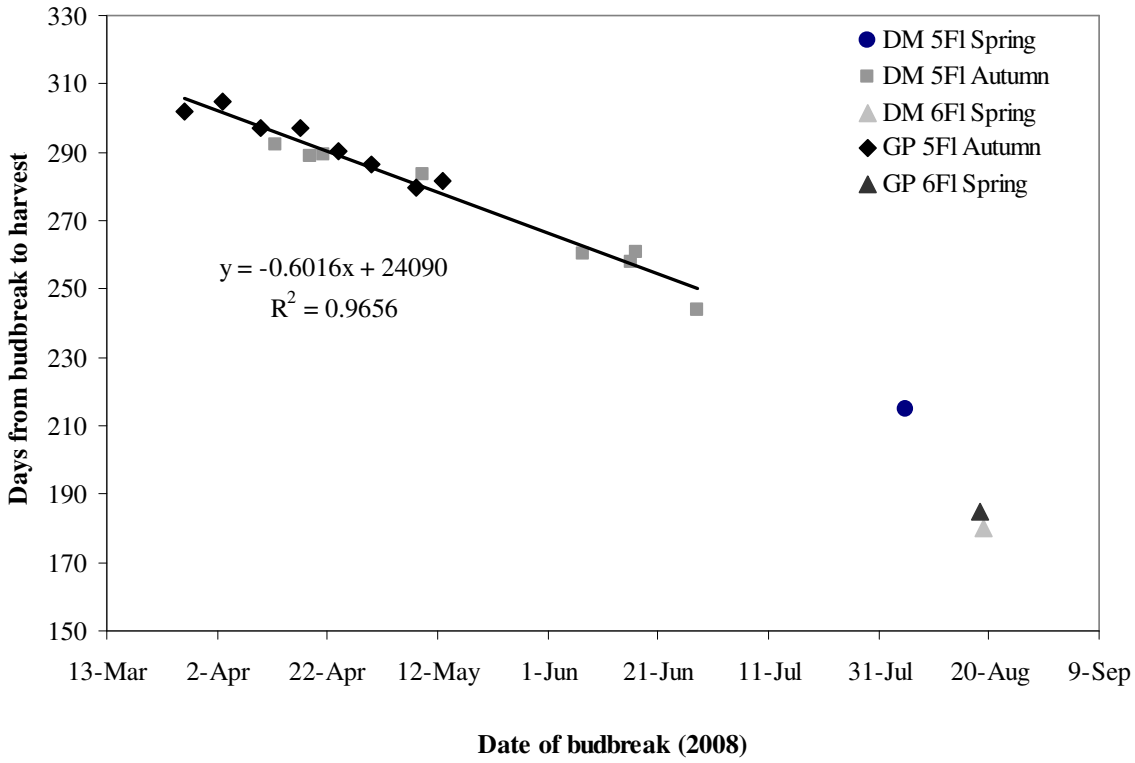
Fig. 5. The percentage (%) of MaxCel™ treated stems harvested before and up to week 6 (6 February 2009) compared to the percentage (%) stems harvested within the commercial production system (CP). Four-flush *Protea* 'Pink Ice' shoots were treated terminally on either dormant or greenpoint buds with MaxCel™ at 500 $mg.L^{-1}$ (active ingredient: 6-benzyladenine (BA) 1.9%) on ten consecutive treatment dates from March to June 2008.



ANOVA

SOURCE	*F value*	*Pr > F*	*Significance*
Treatment	5.89	<.0001	***
Commercial production (CP) vs. treatment	2.34	0.129	ns

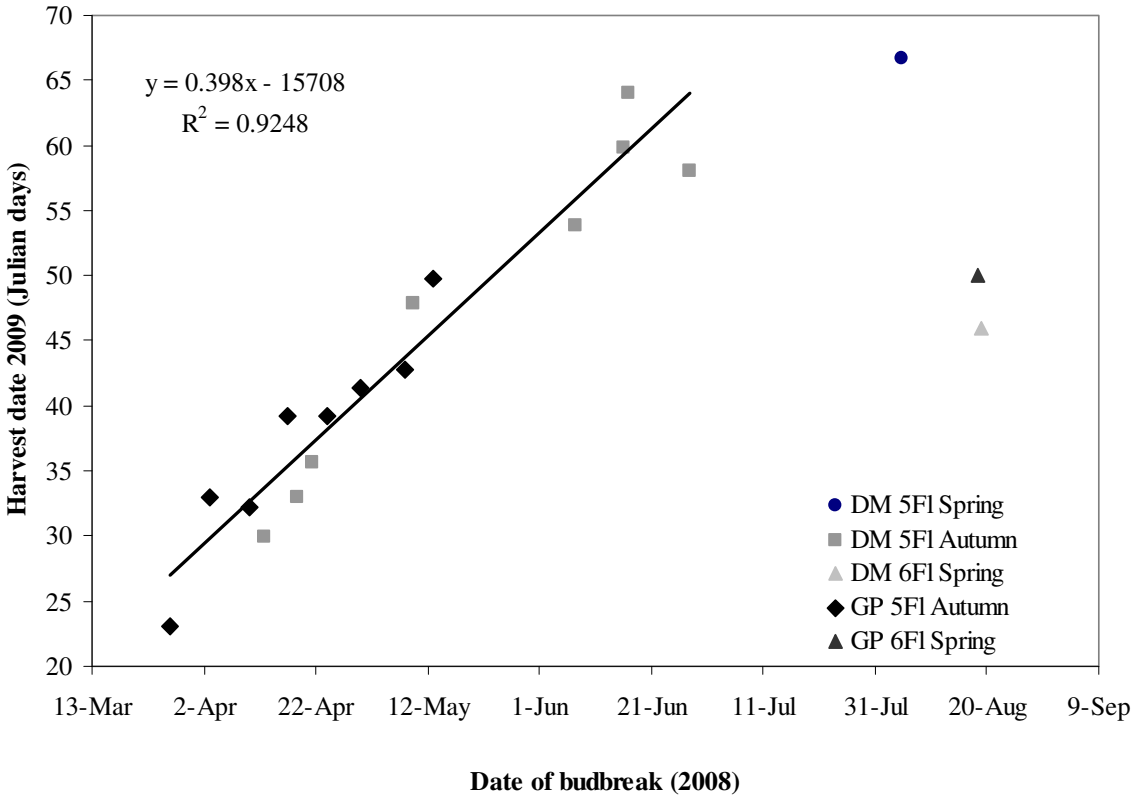
Fig. 6. The percentage (%) flowering recorded on four-flush *Protea* cv. Pink Ice shoots that flushed after weekly treatments with MaxCel^{TM} at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%) when applied in the dormant state during the autumn of 2008 as well as the percentage flowering on shoots that was found to flush unaided (Greenpoint phenological stage) on these treatment dates. The control shoots were selected on 27 March 2008 and did not receive any MaxCel^{TM} treatment.



PROC CORR

SOURCE	*P-value*	*Significance*
Budbreak date vs days GP to harvest	<.0001	***

Fig. 7. The correlation between the number of days from budbreak to the commercially-ready harvest stage and the date of budbreak for five-flush shoots that yielded autumn-initiated inflorescences when four-flush *Protea* cv. Pink Ice shoots were treated either at the phenological greenpoint (GP) or dormant (DM) stage. Shoots were treated terminally weekly during the autumn of 2008, with a MaxCel™ solution at 500 $mg.L^{-1}$ (active ingredient: 6-benzyladenine (BA) 1.9%). Data for flowering shoots that initiated inflorescences on the spring flush of either five- (DM 5Fl Spring) or six-flush (DM 6Fl Spring; GP 6Fl Spring) shoots after winter, are also presented.



PROC CORR

SOURCE	*P-value*	*Significance*
Budbreak date vs harvest date	<.0001	***

Fig. 8. The harvest date (2009) in Julian days as correlated to the date of budbreak of the flushes subtending the autumn-initiated inflorescences of *Protea* cv. Pink Ice shoots when treated terminally at either the dormant (DM) or greenpoint (GP) phenological stage, from 28 March to 2 June 2008, with a MaxCel™ solution at 500 mg.L^{-1} (active ingredient: 6-benzyladenine (BA) 1.9%). Data for flowering shoots that initiated inflorescences on the spring-flush of either five- (DM 5Fl Spring) or six-flush (DM 6Fl Spring; GP 6Fl Spring) shoots after winter, are also presented.

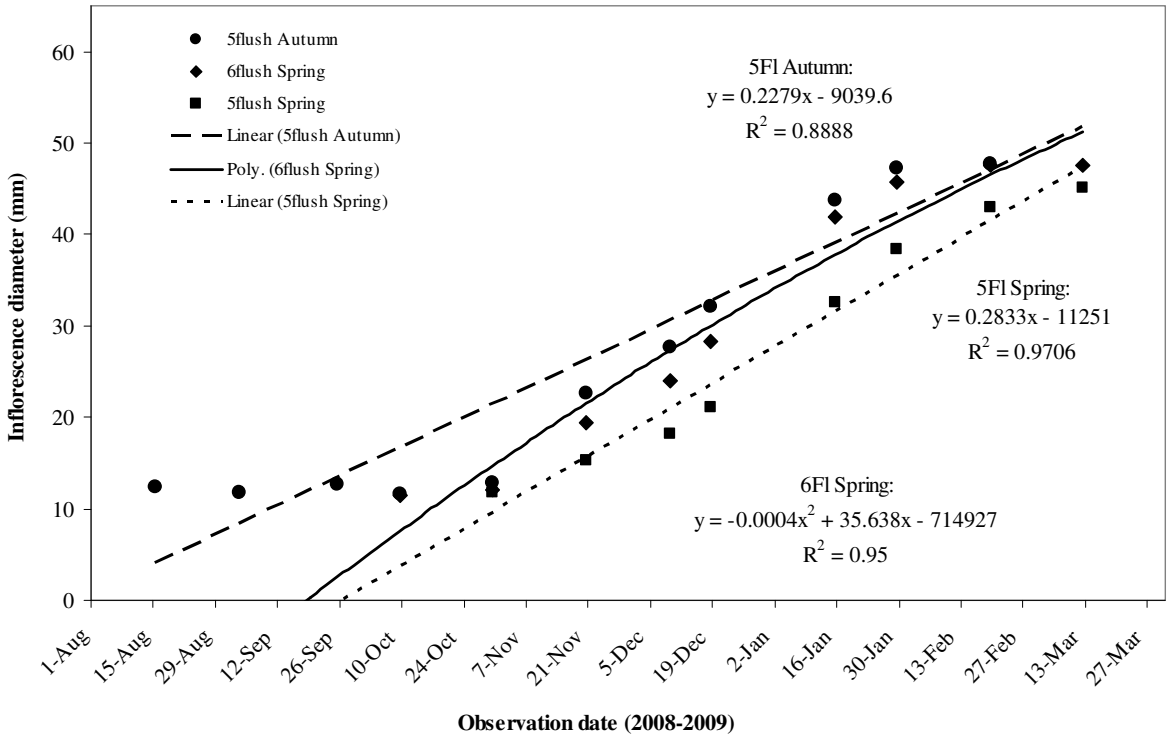


Fig. 9. Inflorescence development of *Protea* cv. Pink Ice expressed as the increase in basal inflorescence diameter (mm) from August 2008 to February 2009, recorded from 10mm size to anthesis. Inflorescences initiated either on a five-flush shoot in autumn or on a five- or six flush shoot in spring, following treatment with a MaxCel™ solution at 500 $mg.L^{-1}$ (active ingredient: 6-benzyladenine (BA) 1.9%) over a ten-week period in the autumn of 2008, from 28 March to 2 June.

Paper II: Managing Vegetative Complexity to Achieve Advanced Flowering Time in *Protea* cv. Pink Ice

Managing Vegetative Complexity to Achieve Advanced Flowering Time in *Protea* cv. Pink Ice

ADDITIONAL INDEX WORDS: Pruning, thinning, shoot synchrony, autumn inflorescence initiation

ABSTRACT. South African produced *Protea* 'Pink Ice' stems flower mostly during the non-profitable marketing months of February to May, even when subjected to a biennial pruning regime. *Protea* producers aim to deliver a superior quality product during the high demand period of September to January to the European markets. The aim of this study was to evaluate various pruning and thinning regimes for its efficacy to advance the flowering time of *Protea* 'Pink Ice' commercially and cost-effectively to the lucrative pre-Christmas period. Mature 'Pink Ice' plants were pruned to yield a final number of 40 marketable stems by varying the combinations of the number of bearers per plant and shoots per bearer. The effects of these various pruning and thinning regimes on the stem quality at harvest as well as harvest times of the subsequent inflorescences were determined. Results concluded that harvests remained spread over a period of 12 months for most treatment combinations, with average harvest dates contained between 20 March and 14 April for all treatment combinations, including the commercial control. The percentage of stems harvested before Valentine's Day did not differ significantly between treatments, nor did the percentage of autumn-initiated inflorescences. The treatment combinations of 20:2 and 13:3 (bearers per plant:shoots per bearer) produced 8% and 11% of its harvest between 28 Nov.-19 Dec. compared to the 0.24% of the harvest recorded for the commercial control in this same period. The treatment combination of 40:1 and 20:2 yielded significantly longer stems compared to other treatments. Still, all treatment combinations produced stems which exceeded 70cm. Harvest week was significantly negatively correlated with stem length at harvest. However, none of the treatment combinations could produce shoots where these superior characteristics could significantly advance flowering time. Only limited success could be achieved to advance flowering by means of pruning to bearers, together with a thinning regime. Therefore, the use pruning and thinning regimes alone as evaluated in this study cannot unlock the plasticity of 'Pink Ice' to flower within the pre-Christmas period.

Introduction

All profitable protea cut flower production systems aim to harvest the highest number of quality stems within a period of high demand. For *Protea* best prices are achieved when long-stemmed blooms can be delivered within the European winter months of September to January. However, almost all commercially produced *Protea* cultivars in South Africa such as 'Pink Ice' (*P. compacta* x *P. susannae*), 'Carnival' (*P. compacta* x *P. neriifolia*), 'Brenda' (*P. compacta* x *P. burchellii*) and 'Susara' (*P. magnifica* x *P. susannae*), naturally flower outside this optimum marketing period (Gerber, 2000). Although floral initiation and the processes that control it are not well-understood, studies on 'Carnival', 'Sylvia' and more recently 'Pink Ice' have shown that pruning can be used as an effective management tool to improve the stem length as well as to shift flowering time (Gerber et al., 1995; Greenfield et al., 1994; Hettasch et al., 1997; Gerber et al., 2001a; Nieuwoudt and Jacobs, 2010).

Protea cv. Carnival is known to flower exclusively on the spring-flush and only occasionally on the first summer flush, with a corresponding harvest time from February to May (Greenfield et al., 1994). Flower initiation, which coincides with the elongation of the terminal vegetative flush, rarely occurs on the autumn and second summer flushes of 'Carnival' (Greenfield et al., 1994). In an annual bearing system where the harvest cut also serves as the pruning cut, the flowering shoot is cut back leaving a 15 to 20 cm bearer from which an axillary bud will sprout in autumn. This one-flush pre-winter growth will be continued by a spring flush from a terminal position. Only two flushes are sufficient for flower initiation to occur, provided initiation takes place on the spring flush (Greenfield et al., 1994). However, flower initiation on such two flush shoots in spring results in flowers with shorter, less marketable stems together with a delayed flowering time (Greenfield et al., 1994; Gerber et al., 1995).

An alternative to the annual bearing system was suggested by Greenfield et al. (1994) and Gerber et al. (1995) where 'Carnival' plants were pruned during the winter months of June or July as part of a biennial management system. In such a system stems were cut back leaving 15-20 cm bearers at harvest in late summer to autumn (Gerber et al., 1995). The regrowth that was likely to occur until late autumn was again pruned back in June or July, thus removing over-wintering flush growth on which floral initiation would be likely to occur after spring budbreak. This strategy permitted an additional growing season as floral initiation cannot proceed on a single,

basal spring flush. As floral initiation is unlikely to occur on any summer or autumn flush of the current growth season, three- to four-flush shoots would over-winter in a vegetative state, whereafter flowering would occur in spring on a vigorous flush of a superior quality shoot, now consisting of four- or five-flushes. By following a biennial pruning system the total yield of harvestable stems as well as overall stem quality were improved, where 95% of stems were reported to be longer than 50cm compared to only 6% of stems in this category, when plants were managed in an annual system (Gerber et al., 1995; Hettasch et al., 1997). In the biennial system harvests are only attained every second year, however the income per plant due to higher numbers of harvestable stems, compensates for the loss of income from non-synchronised, but consecutive annual harvests (Gerber, et al., 1995; Hettasch et al., 1997).

Protea cv. Sylvia (*P. eximia* x *P. susannae*) differs from 'Carnival' in that this cultivar is able to initiate flowers on any flush at any time throughout the year, provided three or more subtending flushes are present (Gerber et al., 2001a). Inflorescence development in 'Sylvia' then follows directly on initiation and the time of anthesis depends on the time of initiation. This open window, characteristic of *Protea eximia*, allows manipulation of plants to flower at a specific time of year. However, despite this open-initiation window stems of 'Sylvia' still initiate flowers more readily on the vigorous spring flush that follows after a period of winter dormancy. A biennial pruning system was thus suggested by Gerber et al. (2001a) to control and direct shoot growth of 'Sylvia' to allow for initiation on the autumn flush. Pruning of 'Sylvia' stems in June or July within a biennial system was recommended to produce inflorescences on long stems which could be harvested in October to December, within the high-value pre-Christmas period.

Protea cv. Pink Ice in South Africa naturally flowers during an extended period from January to September, with peak production from February to August (Nieuwoudt and Jacobs, 2010). Evidently 'Pink Ice' initiates inflorescences similarly to 'Carnival' where initiation takes place predominantly on the spring flush. However, autumn inflorescence initiation in 'Pink Ice' can be achieved by means of a biennial pruning system (Nieuwoudt and Jacobs, 2010). Such autumn-initiated flowers would allow for the majority of the flowers to be harvested in the high price period of December to January. When a biennial pruning system was evaluated for 'Pink Ice' through a series of monthly pruning (Nieuwoudt, 2006), the highest yield of

marketable stems and thus highest income per plant was attained when plants were pruned during June and July. Still, for this pruning time only 25% of stems reached anthesis in the high demand period, with the remaining 75% of stems harvested during the undesirable window from February to May. The highest number of stems with a premium flowering time was the result of pruning in March for 'Pink Ice'. Stems pruned in March produced a spring flush after a period of winter dormancy that initiated either from an axillary position on the bearer or from a terminal position on a subtending flush that was produced from the bearer in autumn. These terminal spring flushes borne on one- or two flush shoots mostly did not have the capacity to flower. If flower initiation did not occur on the 1st summer flush, vegetative flush extension would continue until autumn the following year, when the shoot would consist of six to seven flushes. Those high quality shoots now had a higher propensity to initiate a flower, as was reported in the study by Nieuwoudt (2006). Such autumn-initiated inflorescences required a longer development time to anthesis (14 weeks) due to the cold winter months compared to spring-initiated flowers (10 weeks) (Nieuwoudt and Jacobs, 2010). However, the advantage gained by autumn-initiated flowers by initiating 12-weeks prior to normal spring-initiation, allowed for a six-week advancement in flowering time compared to spring initiated flowers.

Despite this significant advancement in flowering time, autumn-initiated flowers reached anthesis only from December onwards. In addition, only a limited number of stems from a population could flower within this window. As flowering, through regulation of biennial pruning, rarely occurred between June and November, irrespective of the month of pruning, harvest time was still limited between December to May (Nieuwoudt and Jacobs, 2010).

Increased pressure by exporters and retailers alike to use sea freight as an alternative to air freight for increased profitability and product affordability requires a transport time of approximately 21 days from Cape Town to Rotterdam, the Netherlands. This extended travelling time necessitates harvest peaks as early as November, in order to leave sufficient time to allow for transport, auctioning and retail before the Christmas holidays.

Studies by De Swart (1989) and Hoffman (2006) on *Protea* 'Ivy' and 'Carnival', respectively, showed the requirement of a critical minimum length or shoot diameter to be attained before inflorescence initiation can occur. This finding was also confirmed in *Banksia*, a member of the Australian Proteaceae (Fuss et al.,

1992; Sedgley and Fuss, 1992). If the same threshold shoot quality requirement is also to be of critical importance in 'Pink Ice' to initiate inflorescence, especially on non-spring flushes, manipulation of plant complexity through various pruning strategies to optimize the number of bearers per plant and shoots per bearer, may enhance out-of-season flowering percentages.

The aim of this study was to evaluate whether a combination of the number of shoots per bearer and number of bearers per plant will produce stems with the type of characteristics with regard to stem length and diameter that will allow for natural initiation on the autumn flush, without the use of any growth regulators to assist floral initiation. Autumn initiation on superior quality 'Pink Ice' shoots may provide producers with the opportunity to schedule peak productions within the most sought after pre-Christmas period.

Materials and Methods

EXPERIMENTAL SITE. The experimental site was situated on a commercial *Protea* farm in the Hopefield district (33°2′S 18°20′E) at altitude of 31 m, on the west coast of South Africa. The average annual rainfall of approximately 250-300 mm is focussed mainly during the winter months of June to August. The orchard was established on a sandy soil, with plants spaced in double rows, 3 m wide and 1m between plants. Plants were drip irrigated and managed according to established commercial cultivation practices. All plants were pruned in June 2007 according to a biennial bearing system where the orchard was divided into "on-year" (flowering) and "off-year" (vegetative) blocks (Gerber et al., 1995; Nieuwoudt, 2006).

PLANT MATERIAL AND TRIAL DESIGN. Seven year-old *Protea* cv Pink Ice (*P. compacta* x *P. susannae*) plants were pruned according to commercial practice by heading back both flowering and non-flowering shoots to just below the first intercalation, leaving bearing stumps of approximately 15-20 cm, with about 15-20 leaves per bearer, to support vegetative growth (Nieuwoudt, 2006). All plants that contributed to the commercial harvest were pruned to approximately twenty bearers per plant, but with no thinning performed.

Treatments entailed experimental plants to be pruned during June 2007, to various numbers of bearers per plant whereafter bearers were thinned in October 2007, to a range of different numbers of sprouting axillary buds per bearer to obtain a final number of possible flowering stems of between 24 and 48 per plant (Fig. 1).

Bearer number per plant and axillary bud thinning combinations of bearer:sprouting buds included the range of 40:1; 20:2; 13:3; 10:4; 16:2; 12:2 and 24:2 respectively.

The seven different pruning treatments described above were awarded to plants according to a complete randomised design and repeated five times where a single plant represented an experimental plot. Eight shoots were selected for assessment on each plant for all treatments with the exception of the treatment consisting of 13 bearers with three axillary buds left per bearer, where nine shoots were available for selection per plant.

DATA RECORDING AND STATISTICAL ANALYSIS. Bearer diameters (mm) measured at the distal end, along with the corresponding shoot lengths (cm) and positions of the sprouting axillary buds on the bearer, were determined at the first assessment opportunity in November 2007, following the completion of the spring flush. Thereafter, monthly assessments followed where shoot lengths (cm) and shoot diameters (mm) were determined by means of a tape measure and digital calliper, respectively, together with the recording of the progression of the number of flushes. The position of the shoot on the bearer was determined by counting from the most distal axillary bud on the bearer towards the base, where the most proximal bud was assigned the number one position. Yield per treatment was calculated at harvest as the total number of harvested stems, separated into the percentage stems with summer-, autumn- and spring-initiated inflorescences (Fig. 1), whilst flowering time was presented in a harvest distribution table. Assessments made for the pruning combination 40:1 were based on only four plants, due to plant loss. Analysis of variance (ANOVA) was conducted using the PROC GLM procedure (version 9.1; SAS Institute, Cary, NC). Means were separated according to the least significant difference (LSD) test at $P<0.05$. The CORR PROC procedure (version 9.1; SAS Institute, Cary, NC) was used to determine R^2 and *P*-values of correlations. Log transformations were done for data presented as percentages.

Results

HARVEST DISTRIBUTION. The commercial production harvest distribution for 2008/2009 stretched over an eight month period from November 2008 to June 2009, with a peak production of 19.7% and 13.4% within two consecutive weeks in March 2009 (Table 1). Approximately 37% of commercially harvested stems were picked in a late window, from April to June. Furthermore, only approximately 2% of

inflorescences were harvested by the end of January, thus harvests for commercially produced stems occurred predominantly only after 18 months after pruning (Table 1). No stems were harvested for the commercial production during the first 12 months after pruning (Table 1).

Pruning and thinning treatment combinations 10:4, 16:2 and 12:2 resulted in the highest number of stems harvested within approximately 12 months after pruning, with 26, 24 and 21% harvested between June and August 2008, respectively (Table 1). The harvest distribution of flowers which reached anthesis from plants pruned and thinned to 40:1 or 24:2 resulted in 11 and 14% of inflorescences harvested in June to August of 2008, respectively, whilst the flowering peaks were still recorded in March 2009 (Table 1). An extended flowering time from June 2008 to June 2009 was recorded for plants pruned to 20:2 and 13:3 (Table 1). The highest percentage (95%) of harvestable stems was collected from the 20:2 pruning and thinning combination, although a percentage of >80% of harvestable stems were recorded for all treatments (Table 1). The highest number of stems collected in the high demand period originated from plants pruned to 13 and 20 bearers, although this value only represents approximately 10% of the total harvest or even less (Table 1).

No significant differences were obtained between the respective pruning and thinning treatments for the cumulative percentage of the harvest before 6 February in 2009 where harvest percentages ranged between 19% for the 40:1 and 47% for the 24:2 pruning and thinning combinations, respectively (Table 2).

INFLORESCENCE INITIATION. The percentage of autumn-initiated inflorescences harvested from the various pruning and thinning regimes did not differ significantly from each other, despite ranging from 3 to 31% (Table 2). When rated from high to low, the highest number of stems which initiated inflorescences on the autumn flush was recorded for plants that were pruned according to the 13:3 bearers:shoots pruning and thinning combination. This 13:3 treatment combination also produced no stems on which inflorescences initiated on a summer flush (Table 2). The combination of 24:2 bearers:shoots followed that of the 13:3 combination by producing approximately 25% of autumn-initiated inflorescences, whilst 12% of inflorescences for this treatment combination initiated on the summer flush (Table 2).

As with autumn-initiated inflorescences, no significant differences were observed between treatments for percentages in spring-initiated inflorescences,

despite ranging between 53-75% (Table 2). For all treatments more inflorescences initiated on the spring-flush than for either the summer or autumn flushes (Table 2).

The highest number of non-harvested shoots within the 18-24 months after pruning resulted from pruning and thinning combinations of 40:1, 13:3, 16:2 and 12:2 respectively; however the number of stems not harvested did not differ between treatments (Table 2).

SHOOT QUALITIES. The total stem length at harvest differed significantly between treatments (Table 3). Pruning and thinning treatments 40:1, 20:2, 13:3 and 24:2 produced stems which were, on average, longer than 80 cm, whereas stems from the 10:4, 16:2 and 12:2 treatment combinations had stem lengths between 70 and 80 cm (Table 3). Stems resulting from the combinations 40:1 and 20:2 bearers:shoots ratios were significantly longer than any of the other treatment combinations (Table 3). The treatment 10:4 combination produced significantly shorter stem lengths compared to any of the other treatments (Table 3). Furthermore, the treatment combinations of 10:4 and 16:2 resulted in 30-32% of shoots being shorter than 60 cm (Table 3). Nevertheless, no significant differences were obtained when stem lengths of treatments were sorted within the three length categories (Table 3).

Stem length was significantly inversely correlated with the shoot position as well as the harvest time of the shoots (Table 4). No significant correlations were found between bearer diameter and stem length, shoot position or harvest week (Table 4). Significant differences between treatments of the various pruning and thinning combinations were found for both the stem length and stem diameter at harvest for stems that produced autumn-initiated inflorescences (Table 5). However no significant variation in either stem length or diameter between treatment combinations were observed in stems that initiated inflorescences in spring (Table 5). For autumn-initiated shoots, treatments 40:1 and 16:2 resulted in the longest harvested stems, although representative shoots within these categories were limited (Table 5). Stem lengths associated with both spring-initiated inflorescences were generally above the 80 cm export criteria.

Discussion

Protea 'Pink Ice' initiates inflorescences preferably on the spring flush (Gerber et al., 2001b), but can, when subjected to pruning regimes, also initiate flowers outside of the normal spring window as low percentages of autumn-initiated inflorescences were harvested within a biennial pruning system by Nieuwoudt and Jacobs (2010). In this study the commercially harvested inflorescences resulted in peak harvests during March and April, which coincided with the normal harvest time of between February and May when initiation takes place preferentially for most shoots on the spring flush (Table 1). Only a small percentage (<1%) of the total commercially harvested flowers was collected in November to December, which correlates with the time of harvest of inflorescences that initiated in autumn. When considering the various pruning and thinning treatment combinations, only four of the treatment combinations (20:2; 13:3; 12:2; 24:2) resulted in harvests during this period, also at low percentages of between 3-11% (Table 1). For treatment combination 13:3 a low percentage of about 10% of shoots were harvested in the pre-Christmas period, despite that approximately 30% of its inflorescences were observed to initiate in autumn.

Along with spring- and autumn-initiated inflorescences, summer-initiated inflorescences were also formed. These summer-initiated inflorescences were mostly harvested during the low-marketing European summer months of June to August as was evident in the treatment combinations 10:4, 16:2 and 12:2 which resulted in higher percentages of harvests during this period (Tables 1-2). Thus, pruning manipulations by varying the number of bearers and shoots per bearer were largely unsuccessful to advance flowering time into the pre-Christmas period, even when autumn initiation was achieved.

Reducing the number of shoots per bearer was motivated by the assumption that removing less dominant buds lower down the bearer would also remove sinks competing for photosynthates as these more proximal shoots, which are exposed to poorer light intensities and quality, may take longer before contributing actively to the carbon assimilate pool. Also, Nieuwoudt and Jacobs (2010) recommended that to synchronize shoot growth, the number of shoots that would be permitted to develop per bearer be limited after pruning, to only one or two, depending on the number of bearers present on a plant. Furthermore, in a study by Greenfield et al. (1994) where the number of bearers per cm trunk circumference were increased from 1.5 to 3.5 for

six-year-old plants and from 1.0 to 2.5 for two-year-old plants, the productivity per plant was not increased. In fact, the number of flowers decreased significantly in the six-year-old plants, but was not significantly affected in the two-year-old plants. These results and a study by Hoffman and Jacobs (2012) presented some evidence that floral initiation in *Protea* may be reduced when competing with existing flowering shoots as well as developing vegetative flushes on the same plant.

This study therefore could not present proof that varying the number of bearers per plant and shoots per bearer could provide any commercial benefit in terms of advancing flowering or by producing superior shoot quality. The average harvest date of all treatments and the control still varied in a narrow range between end February and April, whilst the percentage of shoots harvested before week 6, prior to Valentine's Day, did not differ significantly between treatments. Also, total percentage shoots harvested was satisfactorily high at >83% for all treatments.

Time of harvest was significantly negatively correlated with the stem length (Table 4). However, the significantly longer shoots as recorded in the treatment combination of 40:1 and 20:2 (Table 3) did not significantly affect flowering time (Table 1), nor did the significantly shorter shoot length exhibited by the 10:4 treatment combinations. In addition, the final product quality in terms of stem diameter and length were not negatively affected by any treatment combination as harvested stem lengths exceeded the required 80 cm for all treatments, with acceptable stem diameters of generally more than 7 mm recorded.

A greater structural complexity will allow for a greater crop bearing potential and is therefore desirable in *Protea* plantings along with shoot synchronisation by means of winter pruning (Jacobs, 2010). Correspondingly, it is thought that the increase in photosynthetic source size (Gerber et al., 2002) associated with increased shoot length and leaf number, together with shoot thickness (De Swardt, 1989; Hoffman, 2006) will promote out-of-season floral initiation as well as enhance the rate of flower development, thereby advancing flowering time. However, in this study none of the treatment combination could provide the majority of the shoots that resulted from these treatments with characteristics that allowed for the significant advancement of flowering time in the pre-Christmas period.

Conclusion

The various pruning and thinning combinations did not result in significantly higher percentages of autumn inflorescence initiation between treatments or a significant advancement of flowering time. 'Pink Ice' persisted to preferentially initiate inflorescences on the spring flush, irrespective of treatment combination so that the majority of stems were still harvested during the late February to end of March. Pruning of *Protea* 'Pink Ice' within a biennial bearing system is still recommended. The efficacy of the pruning and thinning regime of 13 bearers per plant thinned to 3 shoots per bearer warrants further investigation, but then used in combination with benzyladenine (BA) application, to assist inflorescence initiation in autumn.

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Table 1. Harvest distribution of stems from seven-year-old *Protea* cv. Pink Ice plants harvested during 2008-2009, after plants were subjected to various pruning and bud thinning regimes in June and October 2007, respectively. Inflorescences were commercially harvested from June 2008 to June 2009. Values are expressed as percentages of the total harvest. Shaded blocks represent the peak harvest dates (≥15%). The commercial production control represents the harvest distribution of 'Pink Ice' stems as obtained for the commercial crop that was managed according to a biennial pruning system.

					DATE OF HARVEST																
					Harvest weeks of 2008-2009																
					2008		2009														
	Mean harvest date of harvested stems[z]	STDev of mean harvest date (±days)[z]	Number of selected stems	Percentage of harvested stems (%)	31	48 - 51	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15-27
					5 Jun - 01-Aug	28-Nov - 19-Dec	02-Jan	09-Jan	16-Jan	23-Jan	30-Jan	06-Feb	13-Feb	20-Feb	27-Feb	06-Mar	13-Mar	20-Mar	27-Mar	03-Apr	8 Apr - 29 Jun
Commercial production	23-Mar		32332			0.23	0.28	0.08	0.23	0.52	0.70	1.55	1.88	1.74	3.39	8.52	19.95	13.68	9.65	9.31	28.29
Treatment: Bearers per plant:shoots per bearer																					
40:1	12-Mar	23	32	84	11.11						3.70	7.41		7.41	7.41	11.11	11.11	25.93		11.11	3.70
20:2	27-Feb	30	40	95	5.26	7.89	2.63		2.63	2.63	5.26	10.53	2.63	5.26	10.53	18.42	10.53	5.26	2.63	2.63	5.26
13:3	13-Feb	31	45	84	7.89	10.53	2.63	2.63	5.26	5.26	2.63	5.26	7.89	2.63	13.16	13.16	5.26	10.53	2.63	2.63	
10:4	26-Feb	27	40	85	26.47			8.82		5.88		5.88		5.88	2.94	5.88	17.65	14.71		2.94	2.94
16:2	5-Mar	18	40	83	24.24			3.03	3.03					3.03	9.09	24.24	6.06	21.21	3.03	3.03	
12:2	2-Mar	22	40	83	21.21	3.03			3.03			3.03	6.06		12.12	24.24	15.15	3.03		9.09	
24:2	20-Feb	33	40	90	13.89	5.56		5.56		11.11	5.56	5.56		5.56	2.78	22.22	5.56	5.56			11.11

[z]Harvest dates in June and July were excluded in the calculation of mean.

Table 2. The number of stems initiating inflorescences on the autumn-, spring- or summer-flush, along with the cumulative percentage (%) of stems harvested before and up to week 6 of 2009 (6 February), when seven-year-old *Protea* cv. Pink Ice plants were subjected to various combinations of pruning and thinning treatments during June and October 2007, respectively.

Treatment (Bearers per plant:shoots per bearer)	Number of selected shoots	Number of harvested shoots	Percentage of shoots not harvested[z] (%)	Percentage of stems, harvested before week 6 of 2009 (%)	Percentage of Summer-initiated inflorescences (%)	Percentage of Autumn-initiated inflorescences (%)	Percentage of Spring-initiated inflorescences (%)
40:1	32	27	15.6	18.5	6.3	3.1	75.0
20:1	40	38	5.0	36.8	7.5	17.5	70.0
13:3	45	38	15.6	42.1	0.0	31.1	53.3
10:4	40	34	15.0	44.1	20.0	12.5	52.5
16:2	40	33	17.5	30.3	15.0	10.0	57.5
12:2	40	33	17.5	30.3	17.5	5.0	60.0
24:2	40	36	10.0	47.2	12.5	25.0	52.5
F-Value			0.33	0.32	0.98	1.72	0.71
P-Value			0.91NS[x]	0.92NS	0.46NS	0.15NS	0.65NS

[x]NS indicate non-significance at the 5% confidence level.

[z]Shoots not harvested were because of damage by insects and not due to lack of floral initiation.

Table 3. The percentage (%) of harvested stems per stem length category (≤60 cm; 61-79 cm; ≥80 cm) of seven-year-old *Protea* cv. Pink Ice plants which received various combinations of pruning and thinning treatments during June and October 2007, respectively.

Treatment (Bearers per plant : shoots per bearer)	Number of flowering stems	Average final stem length (cm)	% STEMS IN LENGTH CATEGORIES (cm)		
			≤ 60 cm	61-79 cm	≥ 80 cm
40:1	27	87.44a[y]	3.70	11.11	85.19
20:2	38	89.13a	5.26	5.26	89.47
13:3	38	81.09c	7.89	26.32	65.79
10:4	34	70.71e	32.35	11.76	55.88
16:2	33	79.12cd	30.30	6.06	63.64
12:2	33	78.76d	21.21	6.06	72.73
24:2	36	83.75b	19.44	5.56	75.00
F-Value		4.44	2.10	1.66	1.26
P-Value		<0.001[*]	0.087NS[z]	0.171NS	0.307NS

[y]Means within columns followed by the same letter are not significantly different for LSD at 5% confidence level (P=0.05)
[z]NS indicate non-significant at the 5% confidence level.

Table 4. Correlative relationships of shoot characteristics expressed as Pearson coefficients accompanied by *P*-values for seven-year-old *Protea* cv Pink Ice plants after exposure to a range of pruning and thinning regimes in June and October 2007, respectively. Bearer diameter (mm) was measured after thinning in November 2007 while final shoot length (cm) and diameter (mm) were recorded at the last assessment prior to harvest on 15 January 2009. Parameters were correlated with the harvest week and shoot position on the bearer. Values indicated in **bold** are significant at the 5% confidence level.

	Stem length	Shoot diameter	Shoot position	Bearer diameter	Harvest week
Stem length	*	-0.076	-0.296	0.046	-0.052
	*	0.248	**<.0001**	0.490	**<.0001**
Shoot diameter	*	*	-0.213	0.149	0.115
	*	*	**0.001**	**0.024**	0.083
Shoot position	*	*	*	-0.077	-0.079
	*	*	*	0.246	0.233
Bearer diameter	*	*	*	*	0.115
	*	*	*	*	0.083
Harvest week	*	*	*	*	*
	*	*	*	*	*

Table 5. Shoot characteristics at harvest as described by shoot length (cm) and diameter (mm) where inflorescences initiated both in the autumn or spring of 2008 after plants were pruned and thinned to a range of bearers per plant and number of shoots per bearer in June and October 2007, respectively.

	AUTUMN INITIATED INFLORESCENCES			SPRING INITIATED INFLORESCENCES		
Treatment	Stems harvested (n)	Stem diameter (mm)	Stem length (cm)	Stems harvested (n)	Stem diameter (mm)	Stem length (cm)
40:1	1	8.39 a	100 a	24	7.82	89.18
20:2	7	8.44 a	87.89 b	29	7.83	92.98
13:3	13	8.41 a	81.95 b	23	7.94	84.41
10:4	5	8.87 a	86.67 b	21	7.17	78.83
16:2	2	7.97 a	95.00 a	23	7.88	89.43
12:2	3	6.37 b	83.67 b	23	7.72	87.92
24:2	11	8.08 a	88.38 b	20	7.75	86.67
F-value		3.27	4.30		0.53	1.38
P-value		0.04	0.01		0.78 NS[z]	0.26 NS

[y]Means within columns followed by the same letter are not significantly different for LSD at 5% confidence level (*P*=0.05)

[z]NS indicate non-significance at the 5% confidence level.

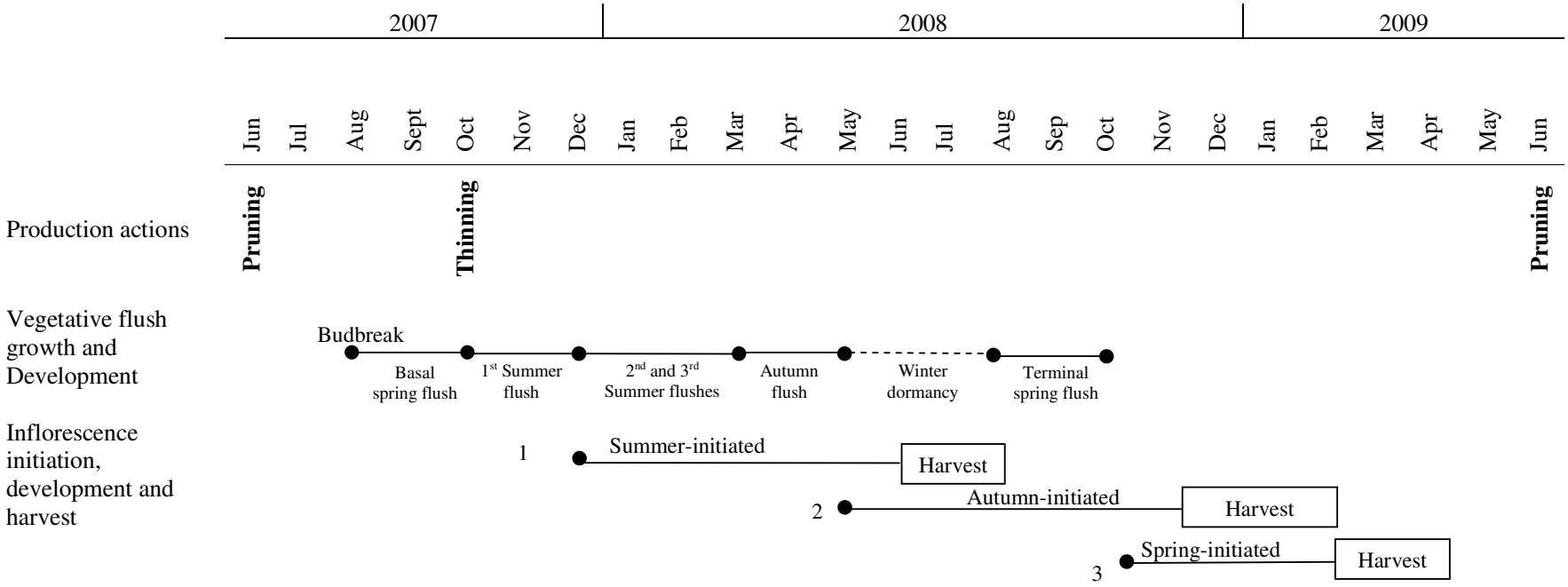

Fig. 1. Time-line representation of the vegetative and reproductive phenology of *Protea* 'Pink Ice' when subjected to a biennial pruning system as was followed for a pruning and thinning trial in June and October 2007 respectively. Vegetative growth phases as well as inflorescence initiation types and subsequent harvests are indicated.

Paper III: The Efficacy of Phenylurea Cytokinin (CPPU) as Alternative Cytokinin Source to Benzyladenine (BA) to Advance Flowering Time in *Protea* cvs. Pink Ice and Carnival

The Efficacy of Phenylurea Cytokinin (CPPU) as Alternative Cytokinin Source to Benzyladenine (BA) to advance flowering time in *Protea* cvs. Pink Ice and Carnival

ADDITIONAL KEY WORDS: Autumn initiation; budbreak; growth regulator; out-of-season harvests

ABSTRACT. South African *Protea* producers are faced with the challenge that most commercially important *Protea* cut flowers are not harvested in a period that coincides with high European market demand, during the Christmas holidays and up to Valentine's Day. The aim of this study was therefore to investigate the efficacy of CPPU (N-phenyl-N'-[2-chloro-4-pyridinyl] urea) as an alternative cytokinin source to benzyladenine (BA) to induce inflorescence initiation in *Protea* 'Carnival' and 'Pink Ice' during autumn. Sitofex™ applications in a concentration gradient that ranged between 1-10 mg.L^{-1} were applied to both 'Pink Ice' and 'Carnival' shoots on 1 April and 16 May 2008 respectively. Applications of BA as MaxCel™ at 500 mg.L^{-1} was made on the April treatment date to both cultivars, whilst a MaxCel™ concentration gradient that ranged from 125-750 mg.L^{-1} was included in the May treatment date for 'Pink Ice' only. The incidence of budbreak and the presence of an autumn-induced inflorescence together with harvest distributions were recorded to evaluate the efficacy of cytokinin applications. In 'Pink Ice', BA applied at 500 mg.L^{-1} together with CPPU at 1 mg.L^{-1} was found to be most successful treatment in initiating high budbreak percentages of between 70-80% when applied in April. Shoots treated with 1 and 5 mg.L^{-1} CPPU in April induced a significant number of autumn-initiated inflorescences so that 81% and 72% of shoots were, respectively, harvested before Valentine's Day. However, none of the treatments with either BA or CPPU in April was successful to advance flowering time for 'Pink Ice' into the pre-Christmas period. The low ability or inability of BA or CPPU to initiate autumn inflorescences in 'Pink Ice' in May were reflected in the relatively late flowering dates which peaked around the last week of February and within the first two weeks of March, with almost no stems being harvested before 6 February. The use of CPPU alone to achieve advanced flowering time in 'Carnival' is not recommended as CPPU for both April and May treatment dates was ineffective to induce autumn inflorescence initiation and advance flowering time. CPPU as an alternative cytokinin source to BA when applied to 'Pink Ice' shoots before May can be considered and warrants further investigation. Future studies should include earlier treatment dates than April in order to achieve early cytokinin-assisted budbreak, with subsequent harvest in the pre-Christmas period.

Introduction

South African produced *Protea* cut flowers are mainly exported to European markets. By exporting to these markets, South African producers have the advantage of an alternate season to the northern hemisphere *Protea* producing countries. However, the flowering time of most of the local commercially available *Protea* cultivars such as 'Carnival', 'Pink Ice', 'Brenda', 'Susara' and 'Lady Di' do not coincide with the period when supply of European grown flowers is low and the demand for niche products are high, as this window includes both Allerheiligen (first week of November) and the Christmas festive season (Gerber, 2000; Hoffman, 2006). Prices obtained during the festive time in Europe can reach double the value that can be obtained for the same product in its normal flowering window, mainly from February to May (Nieuwoudt and Jacobs, 2010), offering significant financial incentives to deliver flowers within this period. *Protea magnifica* as well as the *Protea eximia* hybrid, 'Sylvia', flower within this high demand period. However foliage of these two species are subject to a serious postharvest disorder (leaf blackening) which impacts negatively on product quality and vase life (Windell, 2012). Control of leaf blackening of some species and cultivars can be achieved through glucose pulsing, but an alternative solution is to offer *Protea* selections and varieties with the ability to flower within the high demand period, but with a reduced susceptibility to leaf blackening. The challenge is thus to develop suitable technologies to control inflorescence initiation in *Protea* species and cultivars so that out-of-season flowering or advancement of flowering time can be achieved to meet the European market demand.

Pruning studies conducted on 'Carnival' (Greenfield et al., 1994; Gerber et al. 1995; Hettasch et al., 1997), 'Sylvia' (Gerber et al., 2001a) and 'Pink Ice' (Nieuwoudt, 2006) identified these *Protea* cultivars to display, to a greater or lesser extent, a plasticity in bearing, which would thus allow for the manipulation of flowering time to some degree. However, pruning as a strategy to advance flowering time commercially into the pre-Christmas period to date was largely unsuccessful.

Hoffman et al. (2009) introduced a novel strategy for *Protea* in which the application of exogenous cytokinin (benzyladenine) in autumn in conjunction with a biennial pruning regime could induce early inflorescence initiation on the autumn flush of *Protea* cv. Carnival. Thus autumn-initiated inflorescences can advance the harvest with more than two months compared to the natural flowering window of

shoots that initiate inflorescences in spring. However, the success rate to induce autumn flowering in 'Carnival' by means of benzyladenine application was not consistent (Hoffman, 2006). Reasons for the inefficacy of BA to induce flowering early in the season and the slow release of dormancy of the terminal buds later in autumn remains unclear.

Cytokinins are structurally diverse and biologically versatile compounds that include a large array of natural and synthetic compounds (Mok and Mok, 2001). Natural cytokinin includes adenine derivatives of which kinetin and N^6-benzyladenine (BA) are of the most well-known groups, with ring substitutions at the N^6 position. The phenylureas constitute a group of synthetic cytokinin, some of which are highly active, such as CPPU (N-phenyl-N'-[2-chloro-4-pyridinyl] urea) and thidiazuron (TDZ; N-phenyl-N'-(1,2,3-thidiazo)-5-yl)urea]). For cytokinin, even small substitutions to the N^6 side chain can have pronounced effects on their biological activity. As such, these synthetic analogues of diphenylurea (DPU) such as CPPU and TDZ exhibit cytokinin activity exceeding that of zeatin, a natural cytokinin with an unsaturated isoprenoid side chain (Itai et al., 1995). Also, in contrast to zeatin, these phenylureas are highly stable as they are known to be strong inhibitors of the cytokinin-oxidase enzymes that readily degrade unsaturated N6-iosprenoid side chains, converting active cytokinin such as zeatin to adenine. In addition, the conversion of cytokinin nucleotides to nucleoside, which is not susceptible to cytokinin oxidase, is stimulated by phenylurea cytokinin. These properties, together with the lack of evidence for competition for cytokinin receptors with adenine-based cytokinin, suggest the mechanism of urea-type cytokinin to be via altering endogenous cytokinin metabolism (Christianson and Hornbuckle, 1999; Horgan, 1992).

Cytokinin is known to have effects on many physiological and developmental processes in plants in addition to cytokinesis. These processes include leaf senescence, nutrient mobilization, apical dominance, and the regulation of apical meristem activity, vascular development as well as the breaking of dormancy (Kamínek et al., 1992). The specific biological activities of cytokinin have been exploited for a wide range of applications within commercial horticulture. Cytokinin is extensively used to improve berry or fruit size in persimmon, kiwi, melon, blueberry, avocado, kiwi and grape (Fathi et al., 2011; Hayata et al., 2000; Koron and Stopar, 2006; Latocha and Krupa, 2008; Lovatt, 2005; Reynolds et al., 1992;

Salinero et al., 2007). Other commercial uses of cytokinin range from fruit thinning and increasing return yield (Curry and Greene, 1993; Stern and Flaishman, 2003; Woolley and Currie, 2006), the promotion of fruit set and parthenocarpy (Hayata et al., 1995) to defoliation in cotton and other crops (Suttle, 1986).

Of particular importance for this study, is the role that cytokinin plays along with auxins, in axillary bud sprouting, which is considered a process of apical dominance release (Davenport, 2003, Gaspar et al., 1996; Letham and Palni, 1983; Müller and Leyser, 2011). Urea type synthetic cytokinin such as TDZ and CPPU seem to be more effective at lower concentrations, although still dose-dependent, in inducing budbreak and shoot growth than purine types like zeatin, benzylamino purine (BAP) and kinetin (Christianson and Hornbuckle, 1999; Gaspar et al., 1996; Vinayak et al., 2009). In vitro studies on the release of apical dominance in cultured *Rosa hybrid* confirmed CPPU to exhibit similar physiological effects to BA, but at much lower concentrations (Kapchina-Toteva et al., 2000). In addition, CPPU was also found to be highly effective to overcome the inhibition of the auxin, IBA, with respect to bud sprouting. However, the use of BA to facilitate in vitro shoot proliferation in *Pyrus pyrifolia* was found to be the preferred cytokinin formulation as TDZ and CPPU caused translucent, thick and brittle explants and malformed stomata, a condition known as induce hyperhydricity (Kadota and Noiimi, 2003). Hyperhydricity appeared to be mainly affected by the type of cytokinin, whereas concentration had little influence.

Cytokinin, applied as BA, has been reported to promote shoot initiation in tropical species such as mango and lychee (Davenport, 2000). *Protea* has a similar shoot growth and flushing nature to some tropical and subtropical tree crops, such as the above-mentioned species. In *Protea*, inflorescence initiation coincides with the period of shoot elongation of the subtending flush (Gerber et al., 2001b), therefore out-of-season floral initiation is highly dependent on the ability of the dormant shoot to flush outside the normal vegetative growth window. In a study by Hoffman (2006) applications of BA to mature three-flush shoots of 'Carnival' during early autumn (March) induced high percentages of budbreak, but resulted in low success rates in terms of flowering. By contrast, applications in late autumn (May) resulted in both high bud sprouting and percentages of BA-induced flowering. However, the response time from application to budbreak was significantly extended to later during the season compared to early application times. The longer time required to budbreak,

together with a slower development time for inflorescences during the winter period, significantly reduced the advantage associated with an autumn floral initiation compared to a much later spring initiation time.

In addition to the promotion of lateral bud growth, cytokinin are also considered important promoters of flower initiation in a number of perennial fruit trees such as longan, litchi, mango (Chen, 1985), apple and pear (Bangerth, 2009), as well as in floricultural crops such as *Dendrobium* (Goh, 1979), tuberose (Chang et al., 1999), the Australian wild flower *Boronia megastima* (Day et al., 1995) and grapevine seedlings (Srinivasan and Mullins, 1978). Cytokinin is thought to facilitate floral initiation by directing several of the successive morphogenetic and growth changes which are an integral part of the transition to flowering, rather than exhibiting direct or specific florigenic activity. These morphogenetic changes may involve increased mitotic activity in the apex resulting in increased apex size/volume together with enhanced initiation of bud and leaf primordia under decreased apical dominance (Macháčková et al., 1992; Werner et al., 2001). Furthermore, a triple-receptor *Arabidopsis* mutant, where disruption of cytokinin perception occurred, has been reported to result in a reduced shoot apical meristem, leading to a stunted shoot and little or no flower production (Nishimura et al., 2004). With the application of exogenous cytokinin, floral initiation may be assisted through cytokinin-induced nutrient mobilization where nutrients such as sugars and amino acids are known to be preferentially transported and accumulate in cytokinin-treated tissues (Gan and Amasino, 1995).

In mango, the promotion of flower bud formation and the advancement of flowering time by eight weeks were achieved with the application of BA (Chen, 1985). However, the application of TDZ only released bud dormancy, but did not lead to floral induction (Núñez-Elisea and Davenport, 1995). The mechanism by which cytokinin facilitates floral induction in *Protea* is unknown, although Hoffman et al. (2009) suggest cytokinin to be directly involved as part of the floral signal.

The aim of this study was therefore to investigate CPPU as an alternative cytokinin source to benzyladenine, in both *Protea* 'Pink Ice' and 'Carnival', for its efficacy to induce autumn budbreak in dormant shoots, followed by a subsequent autumn inflorescence initiation and a possible advancement of harvests into the high demand festive season.

Materials and Methods

SITE AND PLANT MATERIAL. Plants from a seven-year-old *Protea* cv. Pink Ice (*P. compacta* x *P. susannae)* orchard near Hopefield (33°2'S 18°20'E) and eight-year-old *Protea* cv. Carnival plants (a natural hybrid, possibly *P. compacta* x *P. neriifolia*) from a commercial *Protea* farm in the Stellenbosch district (33°15'S 19°07'E) were used in this experiment. Both study sites are considered to have a Mediterranean climate with typical cool, wet winters and dry hot summers, but with an average rainfall of 200-300 mm in the Hopefield district compared to an annual rainfall of 600-700 mm for the Stellenbosch district.

Plants in the 'Pink Ice' orchard were spaced in double rows of 3 m wide with 1 m between plants, while plants from the 'Carnival' plantation were planted with a 4 m row spacing and 1-1.5 m inter row spacing. Both orchards were managed according to commercial cultivation practices. 'Pink Ice' and 'Carnival' plants were pruned similarly in winter, according to a biennial pruning regime, to basal bearers of 15-20 cm (Gerber et al., 1995; Nieuwoudt, 2006).

CYTOKININ APPLICATION. Cytokinin applications of Benzyladenine (BA) and N-phenyl-N'-[2-chloro-4-pyridinyl] urea (CPPU) were made by means of a paint brush to dormant, terminal buds of three- and four-flush 'Carnival' and 'Pink Ice' shoots respectively. The concentration range of Sitofex^{TM} applications to 'Pink Ice' and 'Carnival' shoots included an early autumn application date of 1 April 2008 and a later autumn application on 16 May 2008. Sitofex^{TM} (Phenylurea cytokinin/CPPU with active ingredient Forchlorfenuron at 10 mg.L^{-1}; Degussa, Germany) was diluted with distilled water to concentrations of 1, 2, 5 and 10 mg.L^{-1}, respectively. A wetting agent (Silwet L-77®, ABG-7011, Valent Biosciences) was included in the CPPU solution formulation. MaxCel^{TM} (active ingredient 1.9 g.L^{-1} 6-benzyladenine; Valent Biosciences Corporation, Libertyville, Illinois) was prepared at 500 mgL^{-1} (Hoffman et al., 2009) for both 'Pink Ice' and 'Carnival' shoots for the April application date, whereas a concentration range of 125, 250, 500 and 750 mg.L^{-1} BA was applied to only 'Pink Ice' shoots for the application date of 16 May 2008. Thirty shoots were selected for each treatment according to a complete randomized design, where a single shoot represented an experimental unit. Prior to treatment the stem diameter of selected shoots was determined at the upper position of the terminal and the subterminal flush by means of a digital calliper.

DATA RECORDING AND STATISTICAL ANALYSIS. The incidence of budbreak and the presence of autumn-induced inflorescences were recorded to evaluate the efficacy of cytokinin applications. Subsequently, harvest dates were determined to verify harvest advancement and to analyse a harvest distribution. Analysis of variance (ANOVA) was conducted using PROC GLM (version 9.1; SAS Institute, Cary, NC). Means were separated according to the least significant difference (LSD) test at $P<0.05$. Log transformations were done for data presented as percentages.

Results

BUDBREAK. The budbreak incidence of 'Pink Ice' shoots, when treated with a concentration range of CPPU (Sitofex™) in April, resulted in comparable high budbreak percentages at 73% for the 1 mg.L^{-1} CPPU treatment compared to BA-treated (MaxCel™) shoots at almost 80% budbreak (Fig. 1). These budbreak percentages were significantly higher than the budbreak percentages of 50 and 53% which were recorded for the higher CPPU concentrations of 5 and 10 mg.L^{-1} respectively (Fig. 1). The later CPPU applications of 16 May could induce no budbreak in 'Pink Ice', irrespective of concentration.

Budbreak incidences that resulted from BA-treated 'Pink Ice' shoots in May increased with increasing BA concentrations applied, so that the highest budbreak incidences were recorded for shoots treated with 500 and 750 mg.L^{-1} BA at ca. 60% and 87%, respectively (Fig. 2).

In 'Carnival' high budbreak incidences only occurred for CPPU-treated shoots in April, as CPPU largely failed to induce budbreak when applied in May where no or little budbreak was recorded (Fig. 3). For 'Carnival' treated with 500 mg.L^{-1} BA in April, comparable budbreak percentages were recorded to those obtained for CPPU shoots treated at 1 and 5 mg.L^{-1}. However, in the May treatment dates, where CPPU was unsuccessful to achieve commercially meaningful budbreak levels, the BA-treated shoots were able to achieve 100% budbreak (Fig. 3).

HARVEST DISTRIBUTION. 'Pink Ice' shoots treated with CPPU in early autumn (1 April) resulted in a more advanced harvest distribution than shoots treated later in autumn on 16 May (Table 1). For the April treatment dates, CPPU treatments at the lower concentration levels of 1 and 5 mg.L^{-1} resulted in higher percentages of 'Pink Ice' flowers being harvested in late January to early February, approximately 46-47 days earlier than the harvest date of the production control (Table 1). Shoots treated

in April with 10 mg.L^{-1} CPPU resulted in mean harvest dates 17 days later those treated at lower concentrations, on the same date (Table 1). BA-treated 'Pink Ice' shoots at 500 mg.L^{-1} in early-April produced harvests with a peak early February, which was comparable to the harvests of stems treated with the lower concentration CPPU at 1 or 5 mg.L^{-1} on the same date. Shoots treated with 10 mg.L^{-1} CPPU in April only delivered peak harvests by early March, which were comparable to the harvests of the commercial production, although the harvest distribution for the latter was far more evenly spread, from early March until June (Table 1).

For 'Pink Ice' shoots treated with CPPU in May a mean harvest date was generally recorded in the first week of March, with only a small percentage of shoots from the CPPU 2 mg.L^{-1} treatment being harvested in the late November to December period (Table 1). BA- shoots treated in mid-May displayed similar harvest peaks to CPPU shoots treated on the same date, with a distribution that was focussed between the last weeks of February to the second week of March (Table 1).

'Pink Ice' stems treated with a concentration range of BA in May resulted in harvests from late February to mid-March, with an advancement of 21-24 days compared to the commercially harvested flowers (Table 2). The concentration of BA applied did not impact on the harvesting date as similar mean harvesting dates were recorded for the various concentration treatments. However, shoots treated with BA on 1 April at 500 mg.L^{-1} resulted in an earlier mean harvest date, with an advancement of 41 days compared to the commercially harvested stems (Table 2).

'Pink Ice' shoots treated with 1 and 5 mg.L^{-1} CPPU in April led to 72-81% of their inflorescences being harvested before 6 February, in time for Valentine's Day marketing. However, none of the 'Pink Ice' shoots treated in May, either with CPPU or BA, were harvested before this date, with the exception of a few isolated shoots treated with BA at 500 mg.L^{-1}, 2 mg.L^{-1} CPPU and 250 mg.L^{-1} BA (Table 3).

Cumulative flowering percentages just prior to Valentine's Day on 6 February did not differ significantly for 'Carnival' shoots treated in April, irrespective of the source or concentration of the cytokinin (Table 3). Stems of 'Carnival' treated with 500 mg.L^{-1} BA in May resulted in 89.6% of stems being harvested before 6 February in 2009, which was significantly higher than for any other treatment applied in May (Table 3).

INFLORESCENCE INITIATION. For 'Pink Ice' shoots treated in April, the 1 and 5 mg.L^{-1} CPPU applications resulted in the highest number of initiated inflorescences

after sprouting in autumn (Fig. 1), whilst applications with CPPU at 10 mg.L^{-1} lead to significantly lower percentages of autumn-initiated inflorescences, along with stems treated with 500 mg.L^{-1} BA (Fig.1).

The absence of CPPU-induced autumn flushes in May consequently led to no autumn inflorescence initiation recorded for the CPPU treatments made on this date compared to 17% autumn-initiated inflorescences recorded for shoots treated with 500 mg.L^{-1} BA (Fig. 1). When 'Pink Ice' shoots were treated with a concentration range of BA by mid-May, the percentage autumn inflorescence initiation was extremely low, irrespective of BA concentration or the percentage budbreak (Fig. 2).

The highest percentage autumn inflorescence initiation for 'Carnival' shoots resulted from the BA treatment when applied in April and May at 500 mg.L^{-1} (Fig. 3). Low percentages of autumn-initiated inflorescences were observed for 'Carnival' when shoots were treated with CPPU, irrespective of concentration or treatment date (Fig.3).

SHOOT CHARACTERISTICS. Generally, 'Pink Ice' shoots of similar diameter were selected for treatment with CPPU and BA, for both the April and May treatment dates and recorded >7 mm for all treatments (Table 4). For 'Carnival', however, all shoots selected for treatment in April were generally thicker than shoots that were treated in May, irrespective of treatment. However, with the exception of the shoots selected in May for the 5 mg.L^{-1} CPPU treatment, all shoot diameters exceeded 7 mm, immediately prior to application (Table 4).

Discussion

Budbreak in *Protea* is essential for inflorescence initiation to occur since inflorescence initiation coincides with the elongation of the preformed subtending flush. Thus, promoting autumn budbreak is a pre-requisite to ensure the development of autumn-initiated inflorescences, whereby flowering time can be advanced compared to the normal spring initiation window. In this study the efficacy of CPPU as an alternative cytokinin source to BA was investigated, both for its ability to induce budbreak on dormant buds of mature shoots and to initiate inflorescences on the induced autumn flush following application.

'PINK ICE' BUDBREAK AND AUTUMN INLFORESCENCE INITATION- APRIL APPLICATION: In 'Pink Ice', benzyladenine (BA) at 500 mg.L^{-1} together with 1 mg.L^{-1} CPPU was found to be the most successful treatment in initiating high budbreak

percentages of between 70-80% when applied in April (Fig. 1). Budbreak percentages, however, dropped to below 60% when CPPU was applied at concentrations of 5 and 10 mg.L^{-1} on that treatment date. These budbreak percentages are comparable to or lower than the natural budbreak percentage as was recorded in Paper 1, at approximately 74%. Budbreak percentages for 'Pink Ice' did not increase with increasing concentrations of CPPU in April as was reported for 'Pink Ice' shoots treated with a BA concentration range in May in this study (Fig. 2) or for 'Carnival' shoots in a previous study (Hoffman et al., 2009).

Interestingly, the 1 and 5 mg.L^{-1} CPPU treated shoots in April initiated more inflorescences on their induced autumn flushes than that recorded for the BA treatment or the 10 mg.L^{-1} CPPU-treated shoots (Fig. 1). The flowering percentages in the BA-treated shoots were comparable to that of natural autumn-initiation as reported in Paper 1 at approximately 35%, but were reduced in the 10 mg.L^{-1} CPPU treated shoots to below 20 percent. The high percentages of autumn-initiated inflorescences recorded for the 1 and 5 mg.L^{-1} CPPU treated shoots are reflected in the high percentages of inflorescences that were harvested prior to Valentine's Day at 81% and 72%, respectively, compared to only 26-32% for BA- and 10 mg.L^{-1} CPPU-treated shoots (Table 1). However, none of the treatments with either BA or CPPU in April was successful in advancing flowering time into the pre-Christmas period (Table 1).

Increased stem length and diameter have been reported to promote budbreak and autumn flower initiation for 'Carnival' (Hoffman, 2006). 'Pink Ice' shoots selected were mostly of similar diameter, however, an apparently thinner shoot, though not significantly, was selected for the April treatment date with 10 mg.L^{-1} CPPU. The quality of the 10 mg.L^{-1} CPPU shoots could have compromised the ability of this treatment to stimulate budbreak and initiate autumn flowers (Table 4; Fig. 1). However, as the stem diameters of stems treated with 10 mg.L^{-1} CPPU were comparable to those of stems treated with 1 and 5 mg.L^{-1} CPPU where much higher autumn inflorescence initiation could be achieved, the probability of inferior shoot quality that led to low reaction response of budbreak and autumn flower initiation is rejected.

For the April application date on 'Pink Ice' CPPU at the lowest concentration of 1 mg.L^{-1} proved to be equally effective in budbreak to BA. BA, however, was less effective in inducing autumn-initiated inflorescences on the induced flushes than

CPPU-treated shoots, which had similar or lower budbreak percentages at 1 mg.L^{-1} or 2 mg.L^{-1} CPPU, respectively. In *Rosa hybrida* CPPU was reported to be more effective in promoting budbreak at lower concentrations than BA (Kapchina-Toteva et al., 2000), but flowering was not stimulated by either cytokinin-containing growth regulators in *Rosa*. Paper 1 proposed the efficacy of BA to induce autumn inflorescences in 'Pink Ice' to be largely linked to its ability to allow the release of more autumn flushes than would occur with natural budbreak, thereby increasing the opportunity for autumn initiation to take place during flush elongation. For 'Pink Ice' shoots treated with CPPU in April, another mechanism could be active, where the cytokinin stimulates both budbreak and autumn inflorescence initiation at an optimum concentration, as was the case for 'Carnival' where little autumn flowering would occur in the absence of BA, even though budbreak percentage naturally can be high in strong shoots (Hoffman, 2006). Christianson and Hornbuckle (1999) suggested two distinct receptors for BA and CPPU, which would allow for different biological activities and mechanisms of action.

The success of CPPU to initiate autumn-induced inflorescences at higher rates than BA in April-treated shoots may also be linked to the more stable formulation of CPPU compared to that of BA, especially when temperatures prevail which would still permit the rapid and active metabolizing of BA to inactive forms (Itai et al., 1995).

'PINK ICE' BUDBREAK AND AUTUMN INLFORESCENCE INITATION- MAY APPLICATION: The high budbreak percentage of 80% that was achieved for the May application with BA at 500 mg.L^{-1}, was unexpected (Fig. 2) as a comparative study reported in Paper 1 (Fig. 4., Paper 1) could only achieve an average of 20% budbreak with BA application at the same concentration in middle May. However, shoots selected for this study were of a similar diameter (Table 4) than dormant shoots reported in Paper 1 that could achieve budbreak in May with BA application and that succeeded to produce an autumn inflorescence soon thereafter (Table 2, Paper 1). Also, BA-treated shoots in May for this study were of superior quality to shoots reported in Paper 1 that were unsuccessful to induce an autumn inflorescence and which proceeded into winter with a vegetative flush, only to flower in spring. The importance of superior shoot characteristics to ensure responsiveness to BA in terms of budbreak and inflorescence initiation is, thus, again highlighted.

Despite high budbreak percentages of approximately 80% only about 15-18% of the total number of treated 'Pink Ice' shoots could induce an autumn flower with BA application in May (Fig. 2). This low initiation value is similar to that reported in Paper 1 where only 15.6% of total harvested shoots for a comparable treatment date terminated in an autumn-initiated inflorescence (Table 3, Paper 1). Increasing BA concentrations with the later treatment date in May could improve budbreak percentages significantly, but were unable to increase inflorescence initiation (Fig. 2). These low flowering percentages in May for 'Pink Ice' effectively challenge the hypothesis proposed in Paper 1 that BA improves autumn inflorescence initiation by increasing budbreak percentages as increasing budbreak percentages were consistently associated with a similar, low autumn inflorescence initiation.

The low ability or inability of BA or CPPU to initiate autumn inflorescences in May were subsequently reflected in the relatively late flowering dates which peaked around the last week of February and within the first two weeks of March (Table 1), with almost no stems being harvested before 6 February (Table 3).

The effective budbreak and autumn initiation percentages that were obtained by CPPU at lower concentrations in April, could not be repeated with the May application date where no budbreak and thus autumn inflorescence initiation were reported with CPPU, irrespective of concentration. As these shoots were of comparative stem diameter to shoots treated with CPPU in April, with the exception of the shoots that received 10 $mg.L^{-1}$ CPPU, shoot quality was unlikely to be the underlying cause for the lack of response to CPPU in May.

'CARNIVAL' BUDBREAK AND AUTUMN INFLORESCENCE INITATION: Budbreak and autumn inflorescence initiation following application of BA at 500 $mg.L^{-1}$ to terminal buds of dormant shoots of 'Carnival' was comparable for the early April and mid-May BA treatments to that reported for 'Carnival' on comparable dates in 2003 (Hoffman, 2006; Hoffman et al., 2009). In April, CPPU (with the exception of the 2 $mg.L^{-1}$ CPPU treatment), was almost as successful in initiating budbreak in 'Carnival' as the BA treatment. However, when CPPU was applied to dormant 'Carnival' shoots in May budbreak percentages were low or not achieved, similar to what was observed for CPPU application to 'Pink Ice' at this later treatment date. Budbreak and inflorescence initiation percentages for BA treated shoots on the same date, however, peaked at 100% and approximately 90%, respectively (Fig. 3).

CPPU treatments in April across the concentration range were more successful in inducing budbreak in 'Carnival' than with 'Pink Ice' (Fig. 1, 3), except for the CPPU treatment at 2 $mg.L^{-1}$ which was omitted with the April treatment of 'Pink Ice'. The higher budbreak achieved with CPPU in 'Carnival' in April may be ascribed to the more vigorous and continuous flushing nature of 'Pink Ice' compared to that of 'Carnival' where most shoots would already be dormant by 1 April, whilst flushing may still occur in 'Pink Ice' until mid-May. Resting shoots are granted the opportunity to harden off over a longer period and allocate more photosynthetic assimilates to reserve pools rather than to active growth, which may allow for a stronger budbreak in response to cytokinin, as was seen in this study with 'Carnival'.

Autumn-induced inflorescence percentages reported in the April treatments with BA were within the same 35-40% range for both cultivars. These inflorescence initiation percentages were significantly lower in 1 $mg.L^{-1}$ CPPU-treated 'Carnival' shoot applications in April than for BA-treated shoots, opposite to what was recorded for 'Pink Ice' CPPU and BA-treated shoots on the same application date (Fig. 1; Fig. 3).

CPPU was ineffective to induce significant levels of budbreak and thus autumn-initiated inflorescences when applied to 'Carnival' in May. It is plausible that high autumn inflorescence initiation is recorded for 'Carnival' with BA application when the terminal bud is released under BA-induction by late autumn so that shoot elongation takes place under similar inductive conditions which will prevail in spring, when inflorescence initiation preferentially takes place in 'Carnival'. For 'Pink Ice', where no budbreak could be achieved with CPPU in late autumn, these inductive conditions could be not translated into flowering.

The shoot diameters recorded for 'Carnival' treated in May were significantly lower than those selected for treatment in April (Table 4). However, shoot diameters between treatments for the May application date did not differ significantly and could not account for the different response between BA and CPPU treated shoots. Hoffman (2006) proposed a minimum stem diameter of 7 mm for 'Carnival' to have a high propensity to respond to BA in terms of budbreak and autumn inflorescence initiation. This criterion was just met for all 'Carnival' shoots treated in May with average stem diameters ranging between 6.98-7.14 mm. It could be argued that where this threshold of 7 mm may valid for BA at 500 $mg.L^{-1}$, it may be insufficient for

shoots to react to CPPU, irrespective of concentration and may a reason for failure to initiate budbreak with CPPU on the later treatment (Table 4).

Conclusion

The use of CPPU as alternative cytokinin source to BA when applied to 'Pink Ice' shoots before May warrants further investigation. Future studies should include earlier treatment dates than April in order to achieve early cytokinin-assisted budbreak, with subsequent autumn-initiation and harvest in the pre-Christmas period. Since the mechanisms of action of CPPU and BA are thought to be located on different receptors, an application which combines CPPU and BA may yield interesting results. The use of CPPU alone to achieve advanced flowering time in 'Carnival' is not recommended. A better understanding of the mechanism whereby both CPPU and BA induce budbreak and initiate autumn inflorescences is of key importance to unlocking the flowering model for *Protea*.

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Table 1. The harvest distribution of four-flush *Protea* 'Pink Ice' shoots where terminal buds were treated on 1 April and 16 May 2008 either according to a concentration range of 1, 2, 5 and 10 mg.L^{-1} of Sitofex™ (active ingredient: Forchlorfenuron 10 g.L^{-1} (CPPU)) or with MaxCel™ at 500 mg.L^{-1} (active ingredient 6-benzyladenine (BA), 1.9 mg.L^{-1}), respectively. Values are expressed as percentages of the total harvest. Shaded blocks represent the peak harvest dates for a specific treatment date (≥15%). The production control represents the harvest distribution of commercial stems harvested on the same farm.

					DATE OF HARVEST																	
					Harvest weeks of 2008-2009																	
					48 - 51	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17 - 27
Treatment	Harvest date (2009)	STDev (±days)	Harvest adv. (days)[z]	No. of stems	28-Nov - 19-Dec	02-Jan	09-Jan	15-Jan	23-Jan	30-Jan	06-Feb	13-Feb	20-Feb	27-Feb	06-Mar	13-Mar	20-Mar	23-Mar	30-Mar	08-Apr	14-Apr	24-Apr - 29-Jun
Production control	23-Mar	37		32332	0.23	0.28	0.08	0.23	0.52	0.7	1.55	1.88	1.74	3.39	8.52	19.95	13.68	9.65	9.31	3.41	4.98	19.9
Control treatments with MaxCel™ (BA) 500 mgL^{-1}:																						
1 April treatment	10-Feb	17	-41	27			3.70	3.70	3.70	7.41	44.44	11.11	14.81		3.70	3.70			3.70	3.70		
16 May treatment	5-Mar	13	-18	27					3.70				11.11	18.52	33.33	22.22	3.70	3.70	3.70	3.70		
Treatments with Sitofex™:																						
1 April treatment																						
1 mgL^{-1}	4-Feb	22	-47	27		3.70	3.70	11.11	14.81	14.81	33.33	3.70			7.41		3.70				3.7	
5 mgL^{-1}	5-Feb	15	-46	25		4.00			16.00	28.00	24.00	16.00	4.00		4.00			4.00				
10 mgL^{-1}	22-Feb	18	-29	23		4.35			4.35	4.35	13.04	4.35	13.04		43.48	13.04						
16 May treatment																						
1 mgL^{-1}	6-Mar	7	-16	26									3.85	26.92	30.77	34.62	3.85					
2 mgL^{-1}	4-Mar	18	-19	30	3.33								3.33	16.67	50.00	13.33	6.67	6.67				
5 mgL^{-1}	7-Mar	9	-15	29									3.45	24.14	37.93	17.24	13.79		3.45			
10 mgL^{-1}	8-Mar	7	-14	28										17.86	35.71	42.86		3.57				

[z] The advancement of the harvest date of treated shoots was calculated by subtracting the mean harvest date of the treated shoots from the mean commercial peak production date.

Table 2. Harvest distribution of four-flush *Protea* 'Pink Ice' where terminal buds were treated with MaxCel™ at 500 mg.L^{-1} (active ingredient 6-benzyladenine (BA), 1.9 mg.L^{-1}) on 1 April 2008 and with a MaxCel™ concentration range of 125, 250, 500 and 750 mg.L^{-1} on 16 May 2008. Values are expressed as percentages of the total harvest. Shaded blocks represent the peak harvest dates for a specific treatment date (≥15%). The production control represents the harvest distribution of commercial stems harvested on the same farm.

					DATE OF HARVEST Harvest weeks of 2008-2009																	
	Mean harvest date (2009)	STDev (±days)	Harvest adv. (days)[z]	No. of stems	48 - 51 28-Nov - 19-Dec	1 2-Jan	2 9-Jan	3 15-Jan	4 23-Jan	5 30-Jan	6 6-Feb	7 13-Feb	8 20-Feb	9 27-Feb	10 6-Mar	11 13-Mar	12 20-Mar	13 23-Mar	14 30-Mar	15 8-Apr	16 14-Apr	17 - 27 24-Apr - 29-Jun
Production control	23-Mar	37		32332	0.23	0.28	0.08	0.23	0.52	0.7	1.55	1.88	1.74	3.39	8.52	19.95	13.68	9.65	9.31	3.41	4.98	19.9
Control treatments with MaxCel™ (BA) 500 mgL^{-1}:																						
1 April treatment	10-Feb	17	-41	27			3.70	3.70	3.70	7.41	44.44	11.11	14.81		3.70	3.70			3.70	3.70		
Treatments:																						
16-May																						
125 mgL^{-1}	2-Mar	7	-21	28								7.14	3.57	28.57	53.57	7.14						
250 mgL^{-1}	27-Feb	17	-24	29	3.45							3.45	3.45	44.83	34.48	10.34						
500 mgL^{-1}	28-Feb	8	-22	29							3.45	3.45	6.9	48.28	31.03	3.45	3.45					
750 mgL^{-1}	2-Mar	10	-21	30								10	10	26.67	36.67	13.33			3.33			

[z] The advancement of the harvest date of treated shoots was calculated by subtracting the mean harvest date of the treated shoots from the mean commercial peak production date.

Table 3. Cumulative flowering percentage up to week 6 (6 February 2009) of four-flush *Protea* 'Pink Ice' and three-flush *Protea* 'Carnival' shoots when terminal buds were treated on 1 April and 16 May 2008 with a concentration range (1, 2, 5 and 10 mg.L^{-1}) of Sitofex™ (active ingredient: Forchlorfenuron 10 g.L^{-1} (CPPU)) and MaxCel™ at 125, 250, 500 and 750 mg.L^{-1} (active ingredient: 6-benzyladenine 1.9 mg.L^{-1} (BA)). Shoots treated with MaxCel™ at 500 mgL^{-1} were included on each of the treatment dates.

	TREATMENT	
	'Pink Ice'	'Carnival'
1 April 2008 treatment:		
BA 500 mg.L^{-1}	32.00 b[z]	55.56 a
CPPU 1 mg.L^{-1}	81.48 a	34.48 a
CPPU 2 mg.L^{-1}	*	36.67 a
CPPU 5 mg.L^{-1}	72.00 a	53.33 a
CPPU 10 mg.L^{-1}	26.09 b	53.33 a
ANOVA		
F-Value	7.80	1.21
P-Value	0.002	0.338NS
16 May 2008 treatment:		
CPPU 1 mg.L^{-1}	0.00 a	13.33 b
CPPU 2 mg.L^{-1}	3.33 a	10.34 b
CPPU 5 mg.L^{-1}	0.00 a	0.00 b
CPPU 10 mg.L^{-1}	0.00 a	0.00 b
BA 125 mg.L^{-1}	0.00 a	*
BA 250 mg.L^{-1}	3.45 a	*
BA 500 mg.L^{-1}	0.00 a	89.66a
BA 750 mg.L^{-1}	0.00 a	*
ANOVA		
F-Value	0.68	72.60
P-Value	0.690NS	<.0001

* No treatments were performed.

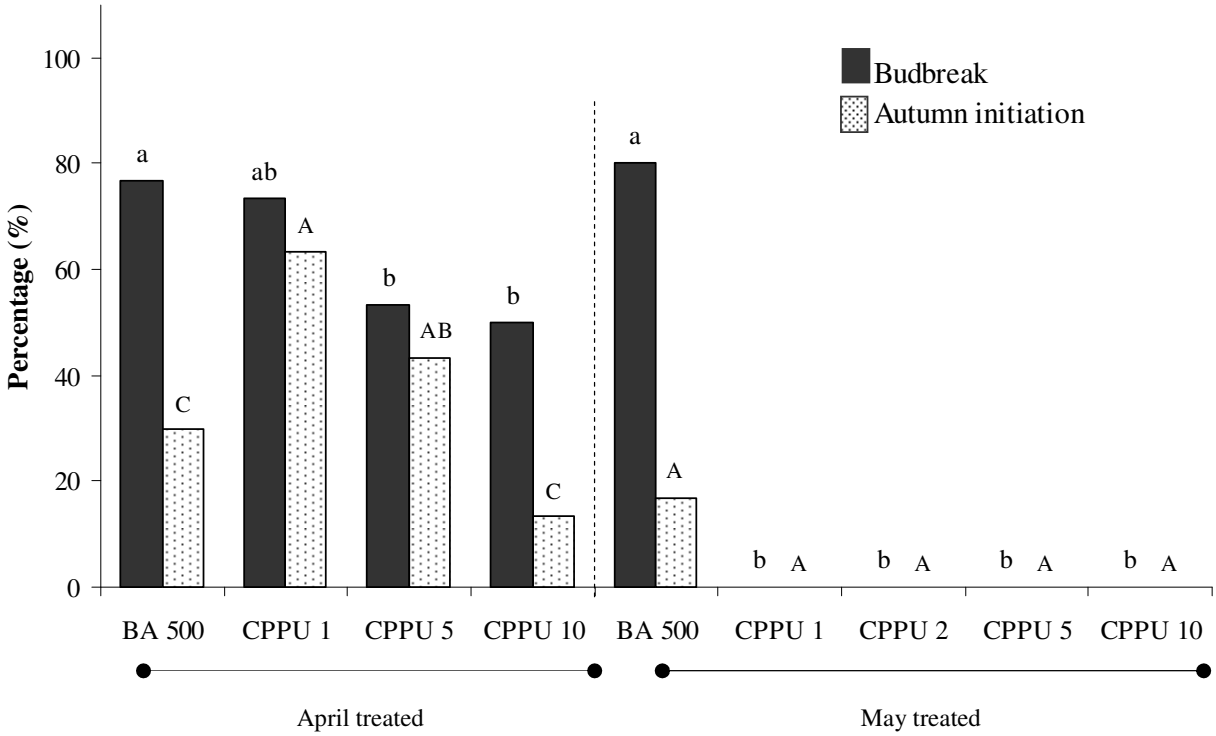
[z]NS indicate non-significant at the 5% confidence level.

Table 4. Stem diameters (mm) as measured at the intercalation of the two subtending flushes to the terminal bud of four-flush *Protea* 'Pink Ice' and three-flush *Protea* 'Carnival' shoots immediately prior to treatment. Shoots of 'Pink Ice' (n=30) were treated with a concentration range (1, 2, 5 and 10 $mg.L^{-1}$) of Sitofex™ (active ingredient: Forchlorfenuron 10 $g.L^{-1}$ (CPPU)) on 1 April and 16 May, whilst shoots treated with a concentration range of MaxCel™ solutions at 125, 250, 500 and 750 $mg.L^{-1}$ (active ingredient: 6-benzyladenine 1.9 $mg.L^{-1}$ (BA)) were included in the May treatment date, but were only applied at 500$mg.L^{-1}$ for the 1 April treatment date. 'Carnival' shoots were treated with CPPU at a similar concentration range to 'Pink Ice', both 1 April and 16 May. Only MaxCel™ applications at 500$mg.L^{-1}$ were included for 'Carnival' shoots on both treatment dates.

	STEM DIAMETER (mm)	
	'Pink Ice'	'Carnival'
1 April treatment:		
BA 500 $mg.L^{-1}$	7.71	7.63
CPPU 1 $mg.L^{-1}$	7.62	7.73
CPPU 2 $mg.L^{-1}$	*	7.52
CPPU 5 $mg.L^{-1}$	7.56	7.52
CPPU 10 $mg.L^{-1}$	7.39	7.65
ANOVA		
F-Value	1.53	0.89
P-Value	0.210NS[z]	0.471NS
16 May treatment:		
CPPU 1 $mg.L^{-1}$	7.81	7.14
CPPU 2 $mg.L^{-1}$	7.7	7.12
CPPU 5 $mg.L^{-1}$	7.88	6.98
CPPU 10 $mg.L^{-1}$	7.82	7.14
BA 125 $mg.L^{-1}$	7.60	*
BA 250 $mg.L^{-1}$	7.77	*
BA 500 $mg.L^{-1}$	7.81	7.12
BA 750 $mg.L^{-1}$	7.71	*
ANOVA		
F-Value	0.97	0.50
P-Value	0.460NS	0.735NS

*No treatments were done.

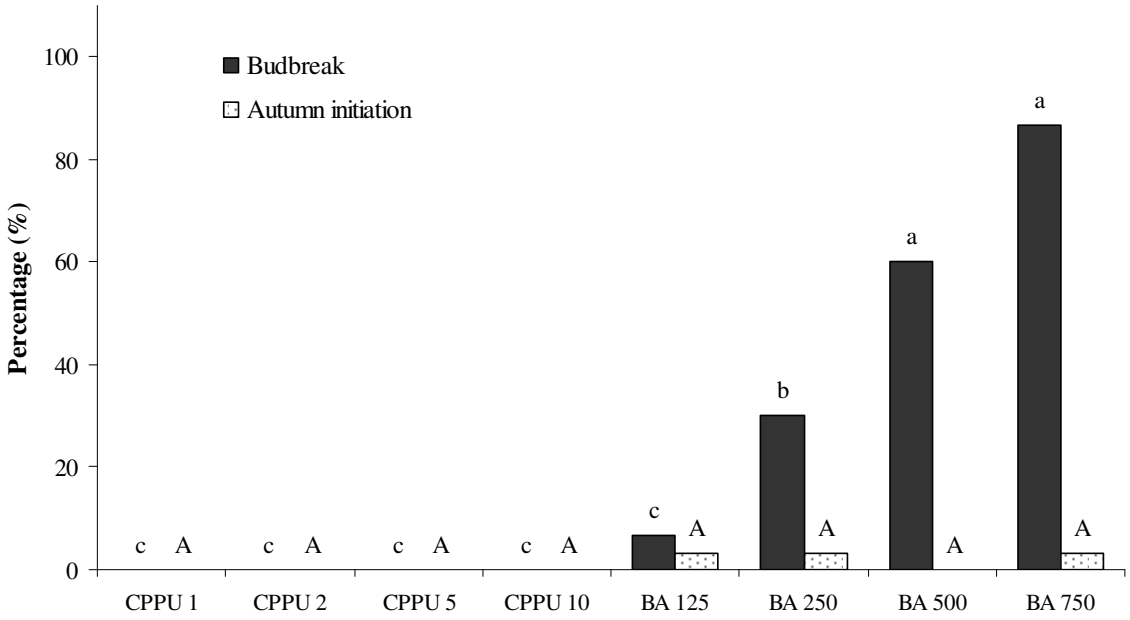
[z]NS indicate non-significant at the 5% confidence level.



ANOVA

SOURCE	*F value*	*Pr > F*
Budbreak – April	3.18	0.052
Budbreak - May	4.46	0.018
Autumn initiation – April	20.69	<0.0001
Autumn initiation - May	0.59	0.676

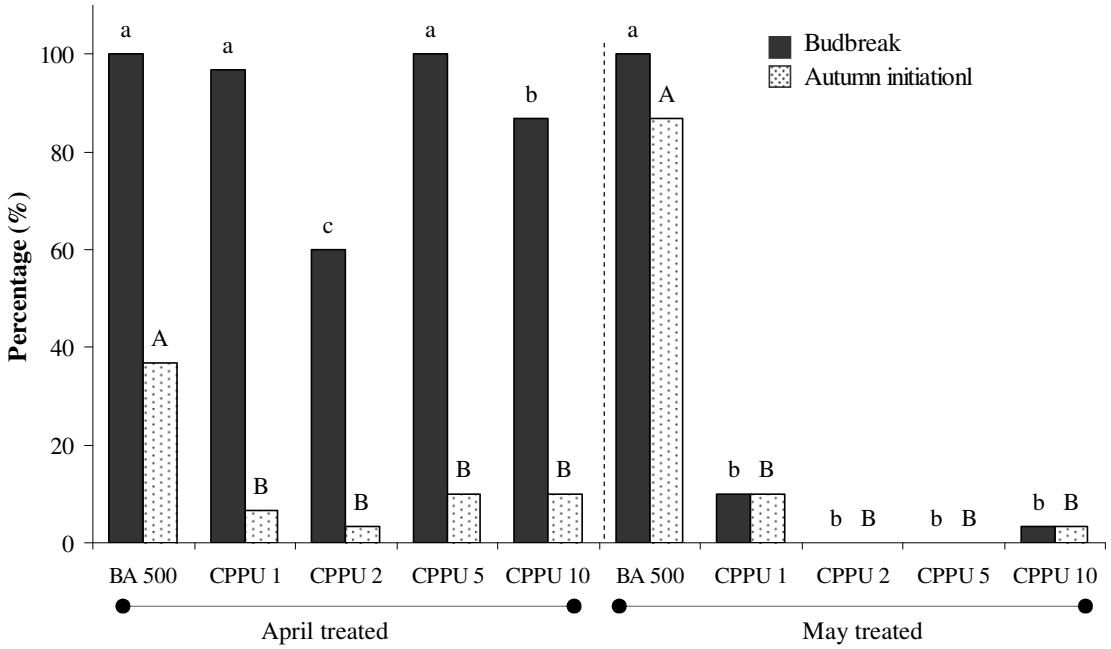
Fig. 1. The percentages (%) of budbreak and autumn inflorescence initiation following treatment of *Protea* 'Pink Ice' terminal buds with a concentration range of 1 (CPPU 1), 2 (CPPU 2), 5 (CPPU 5) and 10 (CPPU 10) mgL^{-1} of Sitofex™ (CPPU) (active ingredient: Forchlorfenuron $10gL^{-1}$) and MaxCel™ (BA) at 500 $mg.L^{-1}$ (active ingredient: 6-benzyladenine at 1.9 $g.L^{-1}$) on 1 April and 16 May 2008, respectively. Letters (lower case letters apply to % budbreak; uppercase letters apply to % autumn initiated inflorescences) that are different indicate significant difference at the 5% confidence level. CPPU at 2 $mg.L^{-1}$ was not included in the April treatments.



ANOVA

SOURCE	*F value*	*Pr > F*
Budbreak	26.73	<.0001
Autumn initiation	0.38	0.905

Fig. 2. The percentage of budbreak (%) and autumn inflorescence initiation (%) following treatment of terminal buds of *Protea* 'Pink Ice' shoots with a concentration range of both MaxCel™ (BA) solution concentrations at 125 (BA 125), 250 (BA 250), 500 (BA 500) and 750 (BA 750) mg.L^{-1} (active ingredient: 6-benzyladenine, 1.9 g.L^{-1}) and Sitofex™ (CPPU) (active ingredient: Forchlorfenuron 10gL^{-1}) at 1 (CPPU 1), 2 (CPPU 2), 5 (CPPU 5) and 10 (CPPU 10) mg.L^{-1} respectively on 16 May 2008. Letters (lower case letters apply to % budbreak; uppercase letters apply to % autumn initiated inflorescences) that are different indicate significant difference at the 5% confidence level.



ANOVA

SOURCE	*F value*	*Pr > F*
Budbreak – April	18.47	<0.0001
Budbreak - May	75.22	<0.0001
Autumn initiation – April	5.19	0.0049
Autumn initiation - May	29.91	<0.0001

Fig. 3. The percentages (%) of budbreak and autumn inflorescence initiation following treatment of *Protea* 'Carnival' terminal buds with a concentration range of 1 (CPPU 1), 2 (CPPU 2), 5 (CPPU 5) and 10 (CPPU 10) mgL^{-1} of Sitofex™ (CPPU) (active ingredient: Forchlorfenuron $10gL^{-1}$) and MaxCel™ (BA) at 500 $mg.L^{-1}$ (active ingredient: 6-benzyladenine at 1.9 $g.L^{-1}$) on 1 April and 16 May 2008 respectively. Letters (lower case letters apply to % budbreak; uppercase letters apply to % autumn initiated inflorescences) that are different indicate significant difference at the 5% confidence level.

General Conclusion

The control of flowering in *Protea* has been an area of research and a challenge for various researchers (Domingues et al., 2010; Gerber, 2000; Gerber et al., 2001; Hoffman et al., 2009; Nieuwoudt and Jacobs, 2010). Most research to date focussed on the use of pruning to manipulate flowering time, resulting in the recommendation of a biennial pruning regime for a number of *Protea* cultivars such as 'Carnival', 'Sylvia' and 'Pink Ice'. A biennial pruning system allows for the synchronisation of shoot growth and the improvement of shoot quality, which, in turn, promotes the propensity for inflorescence initiation and accelerated inflorescence development rate to deliver flowers earlier into more favourable marketing periods. When pruning regimes were applied to 'Pink Ice' a plasticity in bearing was exhibited which allowed for autumn initiation of inflorescences, unlike results obtained for 'Carnival' under similar conditions (Nieuwoudt and Jacobs, 2010). With pruning in March, harvests could be shifted from the usual window of February to May, to December and January, but not on a commercially viable scale. This plasticity in bearing in 'Pink Ice' to initiate inflorescences in autumn when certain required shoot conditions prevail and a strategy to exploit this plasticity to advance flowering time was the topic of interest in this study.

Firstly, the use of benzyladenine on mature 'Pink Ice' shoots to assist with budbreak in early autumn and to initiate inflorescences on these induced flushes was evaluated, following a similar model which proved successful for 'Carnival' (Hoffman et al., 2009), both in terms of enhanced budbreak and advanced flowering time. In 'Pink Ice' the application of benzyladenine in autumn to terminal buds of four-flush shoots increased the percentage earlier budbreak on dormant shoots, though not later in the season, but did not significantly increase inflorescence initiation as was the case in 'Carnival'. Increasing the number of shoots that sprouted in autumn and thereby providing more shoots an opportunity to initiate an inflorescence on this additional flush appears to be the main effect of benzyladenine in 'Pink Ice' in this study. Also, the promotion of additional vegetative flushes on shoots which did not initiate autumn inflorescences decreased the incidence of spring-initiated inflorescences on five-flush shoots. Increasing the size of the source from five to six flushes prior to winter reduced the period from greenpoint to harvest on average, and therefore advanced the harvest date significantly. Still, the use of benzyladenine at 500 $mg.L^{-1}$ within a biennial pruning regime, on 'Pink Ice' could not shift the peak of the harvest time into the required pre-Christmas period.

Subsequently, an alternative cytokinin source to benzyladenine namely a phenylurea cytokinin, CPPU, was explored for its efficacy to promote autumn initiation in comparison to benzyladenine, in both 'Pink Ice' and 'Carnival'. 'Pink Ice' shoots treated with the lower concentrations of CPPU in early-April induced significant numbers of autumn-initiated inflorescences so that between 70-82% of these treated shoots were harvested before Valentine's Day compared to 32% for BA-treated shoots on the same date. However, none of the treatments with either BA or CPPU in April was successful to advance flowering time for 'Pink Ice' into the pre-Christmas period. Further studies in this field are required for 'Pink Ice' and should include treatments earlier than April to allow for harvests to be advanced to earlier than January. However, the use of CPPU to achieve advanced flowering time in 'Carnival' is not recommended as CPPU for both April and May treatment dates was far less effective to induce autumn inflorescence initiation than that which could be achieved with BA application at 500 $mg.L^{-1}$.

Pruning of 'Pink Ice' to various combinations of bearers per plant and shoots per bearer were evaluated, based on previous studies by Nieuwoudt and Jacobs (2010), who recommended further synchronization of shoot growth by controlling the number of shoots that would be permitted to develop per bearer be limited after pruning, to only one or two, depending on the number of bearers present on a plant. However, despite evaluating seven different combinations of bearers per plant and shoots per bearer (40:1; 20:2; 13:3; 10:4; 16:2; 12:2 and 24:2) harvests, irrespective of treatment combination, remained spread over a period of 12 months, with average harvest dates contained between 20 March and 14 April the year following pruning, similar to that of the commercial control. The percentage of stems harvested before Valentine's Day did not differ significantly between treatments, nor did the percentage of autumn-initiated inflorescences.

Although the majority of shoots persisted to initiate inflorescences on the spring flush, the pruning to thinning combination of 13:3 and 20:2 (bearers per plant:shoots per bearer) resulted in an acceptable number of autumn initiated inflorescences. Both these pruning and thinning combinations are suggested to be included in further research, especially as used in conjunction with benzyladenine applications.

The manipulation of flowering time in 'Pink Ice' is possible by means of cytokinin application. Harvests of 'Pink Ice' could possibly be achieved within the pre-Christmas period when cytokinin application is used in combination with a pruning and thinning regime which can effectively improve plant complexity together with shoot quality, and this warrants further research.

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